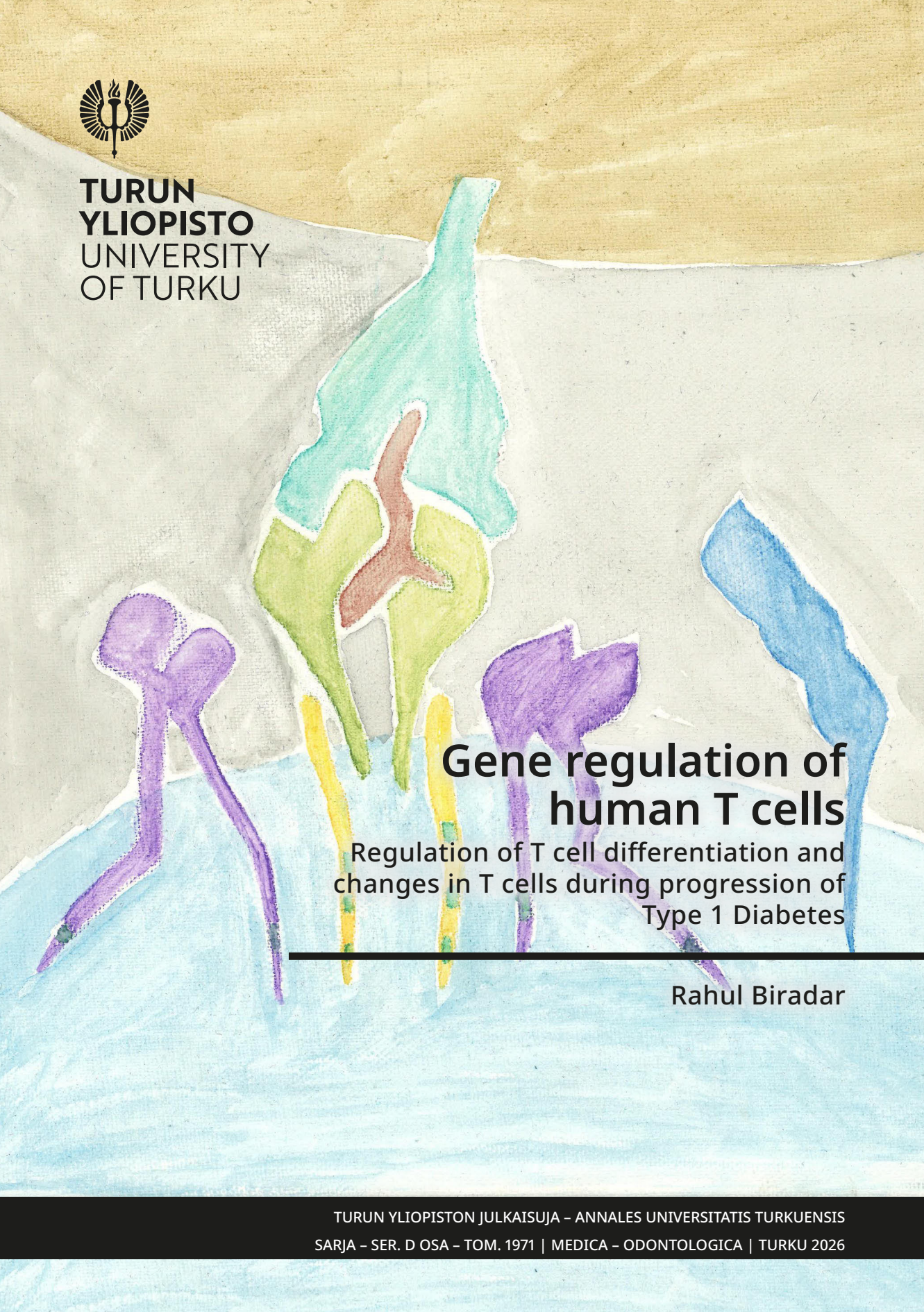

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

# Gene regulation of human T cells

## Regulation of T cell differentiation and changes in T cells during progression of Type 1 Diabetes

Rahul Biradar

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1971 | MEDICA – ODONTOLOGICA | TURKU 2026

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

# GENE REGULATION OF HUMAN T CELLS

## Regulation of T cell differentiation and changes in T cells during progression of Type 1 Diabetes

Rahul Biradar

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1971 | MEDICA – ODONTOLOGICA | TURKU 2026

## University of Turku

Faculty of Medicine
Institute of Biomedicine
Immunology
Turku Doctoral Programme of Molecular Medicine
Turku Bioscience Centre

## Supervised by

Professor Riitta Lahesmaa, MD, Ph.D.
Faculty of Medicine
Turku Bioscience Centre
University of Turku and
Åbo Akademi University
Turku, Finland

Docent Ubaid Ullah Kalim, Ph.D.
Faculty of Medicine
Turku Bioscience Centre
University of Turku and
Åbo Akademi University
Turku, Finland

## Reviewed by

Docent Kirsi Rautajaki, Ph.D.
Faculty of Medicine and Health Technology
Tampere University
Tampere, Finland

Professor Minna Kaikkonen-Määttä, Ph.D.
Faculty of Health Science
University of Eastern Finland
Kuopio, Finland

## Opponent

Professor Lucy Walker, Ph.D.
Division of Infection & Immunity
University college London
London, United Kingdom

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

Cover Image: Rahul Biradar/Drawer

ISBN 978-952-02-0668-0 (PRINT)
ISBN 978-952-02-0669-7 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2026

*Go towards goodness!*

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Immunology
Turku Bioscience Centre
RAHUL BIRADAR: Gene regulation of human T cells: Regulation of T cell differentiation and changes in T cells during progression of type 1 diabetes
Doctoral Dissertation, 145 pp.
Turku Doctoral Programme of Molecular Medicine (TuDMM)
May 2026

## ABSTRACT

CD4+ T cells have emerged as the central players of human adaptive immune system where effector and regulatory T cell subsets regulate various immune responses in health as well as during infections. However, when these immune cells are directed towards bodies own cells/tissues through autoantigen, they initiate autoimmune responses in diseases like rheumatoid arthritis, psoriasis, multiple sclerosis, inflammatory bowls disease and type 1 diabetes.

Type 1 diabetes is chronic autoimmune disease where immune cells kill the insulin producing beta cells of pancreas. Detection of type 1 diabetes associated autoantibodies is the earliest clinical indication of the disease, it also conveys initiation of autoimmune process in pancreas. While disease associated changes have been detected in immune cells at the onset of disease, less is known about T cell changes that contributes to the initiation of the disease. Effector and regulatory CD4+ T cell subsets are associated with the initiation of autoimmunity. Uncovering disease associated changes in specific CD4+ T cell subsets before autoantibodies appear will help to better understand the disease process for possible immune modulation to delay or cure the disease. The work presented in this dissertation report comprehensive and detailed description of single-cell transcriptome of CD4+ T cell subsets from children progressing to type 1 diabetes, before the appearance of type 1 diabetes associated autoantibodies.

The pathogenesis of several autoimmune diseases is linked to proinflammatory effector T cells known as T helper 17 (Th17) cells. Thus, these cells and their key regulatory molecules are being targeted for therapeutic approaches to manipulate Th17 cells in various autoimmune disease. The work presented in the dissertation uncovers new regulators of Th17 cell differentiation. The results indicate both FOSL1 and FOSL2 act synergistically and compete for the binding at BATF occupied loci of the key Th17 genes thus regulating Th17 differentiation. Moreover, epigenetic mechanisms regulate gene expression and the differentiation of Th17 cells. Our results show that a proximal enhancer present in RORA gene regulates Th17 cell differentiation.

KEYWORDS: CD4+ T cells, Autoimmune diseases, Type 1 diabetes, gene regulation, Epigenetic regulation, enhancers, Th17 cells

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Immunologia
RAHUL BIRADAR: Ihmisen T-solujen geenisäätely: T-solujen erilaistumisen säätely ja T-soluissa tapahtuvat muutokset tyypin 1 diabeteksen puhkeamisen aikana
Väitöskirja, 145 s.
Molekyylilääketieteen tohtoriohjelma (TuDMM)
Toukokuu 2026

## TIIVISTELMÄ

CD4+ T-solut ovat keskeisiä toimijoita ihmisen adaptiivisessa immuunijärjestelmässä, jossa efektori- ja säätely-T-solualatyypit säätelevät erilaisia immuunivasteita sekä terveessä elimistössä että taudinaiheuttajainfektioiden aikana. Kun nämä immuunisolut kuitenkin kohdistuvat elimistön omiin soluihin autoantigeenien välityksellä, ne käynnistävät autoimmuunireaktioita, jotka voivat johtaa autoimmuunisairauksiin, kuten nivelreumaan, psoriaasiin, multippeliskleroosiin, tulehduksellisiin suolistosairauksiin ja tyypin 1 diabetekseen.

Tyypin 1 diabetes on krooninen autoimmuunisairaus, jossa immuunisolut tuhoavat haiman insuliinia tuottavat beetasolut. Vaikka haimaperäisten autovasta-aineiden havaitseminen on sairauden varhaisin kliininen merkki, se kertoo myös autoimmuuniprosessin käynnistymisestä haimassa. Koska CD4+ T-solut koordinoivat immuunivasteita, niissä havaitaan tautiin liittyviä muutoksia. On kuitenkin tarpeen ymmärtää ne muutokset, jotka ovat peräisin tietyistä CD4+ T-solualatyypeistä jo ennen autovasta-aineiden ilmaantumista. Tässä väitöskirjassa esitetty työ tarjoaa kattavan ja yksityiskohtaisen analyysin CD4+ T-solualatyyppien yksisolutason transkriptoomista.

Tulehdusta edistävä T-solupopulaatio, joka tunnetaan T-auttaja 17 - (Th17) soluina, osallistuu useiden autoimmuunisairauksien patogeneesiin. Näin ollen näitä soluja ja niiden keskeisiä säätelymolekyylejä tutkitaan uusien terapeuttisten lähestymistapojen kehittämiseksi Th17-solujen toiminnan muokkaamiseksi. Väitöskirjassa esitetty työ paljastaa Th17-erilaistumisen uusia säätelijöitä geneettisten ja epigeneettisten mekanismien kautta. Tulokset osoittavat, että sekä FOSL1 että FOSL2 toimivat synergiassa ja kilpailevat sitoutumisesta BATF:n miehittämiin keskeisten Th17-geenien kohtiin ja säätelevät siten erilaistumista. Lisäksi epigeneettiset mekanismit, jotka liittyvät tehostajajaksojen (enhancerien) kautta tapahtuvaan geenikespression säätelyyn, voivat vaikuttaa Th17-solujen erilaistumiseen. Tulokset osoittavat, että RORA-geenin sisällä sijaitseva tehostaja säätelee Th17-erilaistumista.

AVAINSANAT: CD4+ T-solut, autoimmuunisairaudet, tyypin 1 diabetes, geenien säätely, epigeneettinen säätely, tehostajat, Th17-solut

# Table of Contents

# Abbreviations

| | |
|---|---|
| AAV | adeno associated virus |
| Aab | autoantibody |
| AP-1 | activating protein 1 |
| APC | antigen presenting cell |
| ATAC-seq | assay for transposases accessible chromatin using sequencing |
| BATF | basic leucine zipper ATF-like transcription factor |
| BCL-6 | B cell lymphoma 6 |
| CD | cluster of differentiation |
| ChIP-seq | chromatin immunoprecipatation with DNA sequencing |
| CRISPR | clustered regularly interspaced short palindromic repeats |
| DAPA | DNA affinity precipitation assay |
| DC | dendritic cell |
| DE | differentially expressed |
| DKA | diabetic ketoacidosis |
| DKD | double knock down |
| DNA | deoxyribonucleic acid |
| DOE | double over expression |
| EAE | experimental autoimmune encephalomyelitis |
| ELISA | enzyme linked immunosorbent assay |
| eQTL | expression quantitative trait loci |
| FC | fold change |
| FDR | false discovery rate |
| FOSL | FOS like |
| FOXP3 | forkhead box P3 |
| GADA | glutamic acid decarboxylase autoantibody |
| gRNA | guide RNA |
| GSEA | gene set enrichment analysis |
| GWAS | genome wide association studies |
| H3K | Histone 3 lysine |
| HDAC | histone deacetylase |
| HIF-1α | hypoxia inducible factor 1α |

| | |
|---|---|
| HLA | human leukocyte antigen |
| IA2 | insulinoma associated antigen 2 |
| IAA | insulin autoantibody |
| IBD | inflammatory bowel disorder |
| IL | interleukin |
| ILC | innate lymphoid cell |
| IFN | interferon |
| Ig | immunoglobulin |
| KD | knock down |
| LME | linear mixed effect |
| MHC | major histocompatibility complex |
| NK | natural killer |
| NT | non-targeting |
| OE | over expression |
| PBMC | peripheral blood mononuclear cells |
| RNA | ribonucleic acid |
| RORA | RAR related orphan receptor alpha |
| ROTS | reproducibility optimized test statistic |
| SCENIC | single cell regulatory network inference and clustering |
| scRNA-seq | single Cell RNA sequencing |
| SE | super enhancer |
| siRNA | small interfering RNA |
| SLE | systemic lupus erythematosus |
| SNP | single nucleotide polymorphism |
| STAT | signal transducer and activator of transcription |
| T1D | type 1 diabetes |
| TCR | T cell receptor |
| TF | transcription factor |
| TFBS | transcription factor binding site |
| Tfh | T follicular helper |
| TGF-β | transforming growth factor beta |
| Th | T helper |
| TNF | tumor necrotic factor |
| Tregs | T regulatory cells |
| TSS | transcription start site |
| UMAP | uniform manifold approximation and projection |
| ZnT8 | zinc transporter 8 |

# List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

*I.* **Biradar R**, Kalim UU, Lönnberg T, Junttila S, Suomi T, Norman SZ, Starskaia I, Paulin N, Vaarala O, Rasool O, Knip M, Elo LL*, Lahesmaa R*. Single-cell RNA-seq analysis of longitudinal CD4+ T-cell samples reveals cell-type specific changes during early stages of type 1 diabetes. *Genome Medicine. 2025 Dec 29;17(1):154.*

*II.* Shetty A*, Tripathi SK*, Junttila S*, Buchacher T*, **Biradar R**, Bhosale SD, Envall T, Laiho A, Moulder R, Rasool O, Galande S, Elo LL, Lahesmaa R. A systematic comparison of FOSL1, FOSL2 and BATF-mediated transcriptional regulation during early human Th17 differentiation. *Nucleic Acids Research. 2022. May 20;50(9):4938-4958.*

*III.* Kalim UU*, **Biradar R***, Junttila S, Khan MM, Tripathi S, Khan MH, Smolander J, Kanduri K, Envall T, Laiho A, Marson A, Rasool O, Elo LL, Lahesmaa R. A proximal enhancer regulates RORA expression during early human Th17 cell differentiation. *Clinical Immunology. 2024 Jul 1;264:110261.*

*Authors contributed equally

# 1 Introduction

Autoimmune diseases represent a major global health challenge, arising from the breakdown of immune tolerance and the inappropriate activation of effector pathways. Among the diverse immune cell subsets implicated in these disorders, CD4+ T cells have emerged as central players due to their potent pro-inflammatory functions and their ability to destabilize tolerance networks. CD4+ T cells are categorized into several subsets, including T helper (Th) 1, Th2, Th17, and regulatory T cells (Tregs), each exerting important roles in the modulation of immune responses. The balance between effector CD4+ T cells and Tregs is crucial for maintaining immune homeostasis, and its disruption—through excessive pro-inflammatory activity or dysfunctional regulation—can drive autoimmune pathology. Thus, understanding CD4+ T cell biology offers therapeutic opportunities to balance immune regulation and prevent or treat autoimmune disease.

Type 1 diabetes (T1D) is a chronic autoimmune disorder, where immune cells progressively destroy insulin producing pancreatic β-cells. Early immune activation, detectable even before autoantibody seroconversion, drives disease progression through dysregulated CD4+ T cell responses. Transcriptomic profiling of CD4+ T cells has revealed gene expression signatures preceding seroconversion, highlighting their potential as early biomarkers of autoimmunity. Among effector subsets, Th17 cells—defined by interleukin-17 secretion—are strongly implicated in autoimmune pathology, yet the regulatory mechanisms governing their differentiation in humans remain incompletely understood. Dissecting the transcriptional and epigenomic programs that drive CD4+ T cell polarization toward the Th17 lineage is therefore critical for clarifying their role in autoimmunity.

In the work presented in this dissertation, whole genome wide sequencing methods were utilized to study transcriptomic changes in CD4+ T cells during T1D progression and to investigate early differentiation of CD4+ T cells into Th17 cells. Specifically, the thesis addresses three aims: (1) to define transcriptomic changes in blood CD4+ T cells prior to seroconversion during T1D progression (2) to investigate the role of FOS-like transcription factors (FOSL1 and FOSL2) in shaping CD4+ T cell polarization towards the Th17 lineage and (3) to identify distal

regulatory regions, such as enhancers, activated during CD4+ T cell polarization toward the Th17 lineage.

Together, these studies provide an integrated view of the transcriptional and regulatory programs shaping CD4+ T cell behavior in T1D and advance our understanding of mechanisms underlying autoimmune progression.

# 2 Review of the Literature

## 2.1 Immune system

The origin of host defense against the invading foreign pathogens can be seen as early as in the single cell prokaryotes, which rely on restriction enzymes and clustered regularly interspaced palindromic repeats (CRISPRs) for self-protection (Gao et al., 2020). In contrast, eukaryotic organisms have evolved a wide range of additional and increasingly sophisticated defense mechanisms. In mammals, the immune system represents a highly complex and coordinated network that protects the host from diverse pathogenic threats, including bacteria, fungi, parasites, viruses, and even aberrant self-derived cancer cells. Through a wide variety of specialized components and processes, the immune system continuously monitors the internal environment and responds to potential disruptions to the body's integrity. A central feature of this system is its ability to discriminate between "self" and "non-self," ensuring that harmful invaders and abnormal cells are targeted while healthy tissues remain unharmed. This discrimination relies on intricate signaling pathways and interactions among a diverse set of immune cells with highly specialized functions (Marshall et al., 2018; Owen et al., 2018).

Immune cells in blood originate from hematopoietic stem cells (HSCs), which reside in the bone marrow and give rise to multipotent progenitors that are highly proliferative. Through the process of hematopoiesis, HSCs divide into multipotent progenitors of myeloid or lymphoid lineage, giving rise to the major branches of the innate and adaptive immune systems, respectively (Figure 1). Additionally, multipotent progenitors give rise to Megakaryocyte/erythrocyte progenitors that produce blood platelets and red blood cells (Anderson et al., 2021). Common myeloid progenitors (CMPs) can differentiate into monocyte-dendritic progenitors or granulocyte-macrophage progenitors. The granulocyte-macrophage progenitors give rise to granulocytes such as eosinophils, basophils and neutrophils as well as distinct monocytes that can differentiate into macrophages. Common lymphoid progenitors (CLPs) differentiate into B lymphocytes and T lymphocytes as well as natural killer cells and innate lymphoid cells (Owen et al., 2018). Lineage imprinting and transcriptional priming is implicated to maintain possibility of lineage plasticity in hematopoietic progenitor biology. The modern single cell RNA sequencing

technologies can resolve these cellular compositions and discover new functionally distinct cell populations that are crucial to mount immune responses.

When a pathogen invades the body, the immune system is activated through a series of finely tuned responses. These responses can be categorized into two main types: innate and adaptive immune responses. Innate immunity is the body's first line of defense and provides a rapid, non-specific response to pathogens. It involves physical barriers, such as the skin and mucous membranes, as well as various immune cells that recognize and attack a wide range of pathogens.

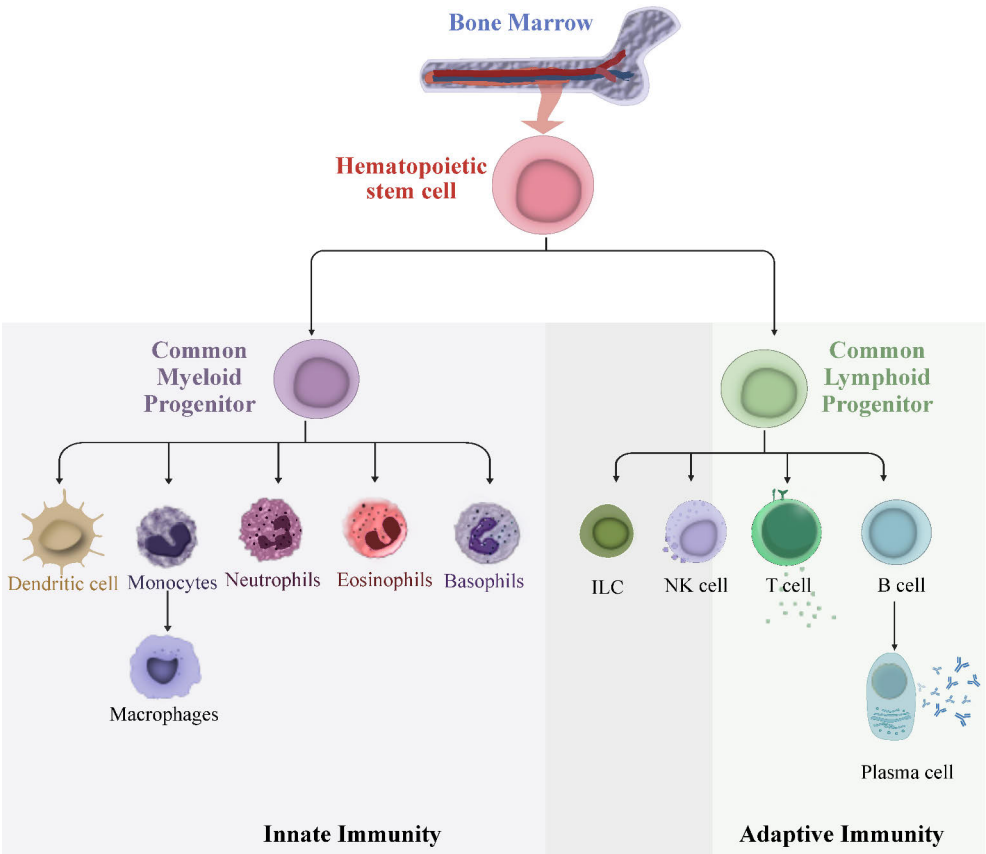


**Figure 1.** Overview of cells of human immune system. Hematopoiesis in bone marrow give rise to various innate and adaptive immune cells in blood.

Adaptive immunity, on the other hand, is more specialized and involves the recognition of specific antigens presented by pathogens. This system generates a targeted response through the activation of lymphocytes, including T cells and B cells. T cells are responsible for identifying and destroying infected or abnormal cells, as well as help B cells to produce antibodies that neutralize pathogens or mark

them for destruction. The adaptive immune system's ability to learn and remember previous encounters with pathogens is another key aspect of its function. This immunological memory allows for a faster and more effective response upon subsequent exposures to the same pathogen, providing long-term protection and the basis for vaccination strategies (Marshall et al., 2018; Owen et al., 2018).

Overall, the immune system's complex and dynamic nature enables it to effectively protect the host organism from a wide array of threats, maintaining health and preventing diseases.

### 2.1.1 Innate immune system

Innate immunity acts as the body's first line of defense against invading pathogens and deploys rapid immune mechanisms to protect the host. Because this response is fast and relies on broadly similar mechanisms against a wide range of pathogens, innate immunity is often referred to as the *non-specific* immune system. The innate immune system includes physical and anatomical barriers (such as the skin and mucous membranes), soluble mediators such as secreted proteins, innate immune cells, and their pattern-recognition receptors (PRRs) (Marshall et al., 2018).

The skin and mucous membranes form a physical barrier at the external and internal surfaces of the body. They also provide chemical protection through enzymes, acids, and mucus that inhibits bacterial and viral growth in the tissue. When pathogens breach these barriers and infect tissues, innate immune cells are rapidly activated to eliminate this threat.

Innate immune cells are primarily derived from myeloid progenitor cells (Figure 1). These include granulocytes, monocytes (which can differentiate into macrophages), dendritic cells and mast cells. Natural killer (NK) cells and innate lymphoid cells (ILCs), although lymphoid in origin, are also considered part of innate immunity due to their lack of antigen-specific receptors. All innate immune cells recognize pathogens through PRRs that bind pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) (Mashall et al., 2018; Owen et al., 2018).

#### 2.1.1.1 Granulocytes

Granulocytes comprise neutrophils, eosinophils, and basophils. These short-lived, phagocytic cells contain granules filled with antimicrobial enzymes and toxins that migrate rapidly to the sites of inflammation (Owen et al., 2018).

**Basophils** store histamine and heparin, contributing to vasodilation and immune cell recruitment. They express high-affinity IgE receptors, which upon allergen-induced IgE crosslinking triggers basophil activation and cytokine secretion, including IL-4 and IL-13, thus promoting allergic inflammation (Owen et al., 2018).

**Eosinophils** contain cytotoxic granule proteins such as lysozymes and play essential role in defense against parasitic infections and in allergic diseases. Their activation can also contribute to tissue damage and amplify inflammatory responses (Owen et al., 2018).

**Mast cells**, although tissue-resident, share functional similarity with basophils. Their granules contain histamine and proteases, and they participate in allergic responses and inflammation within the skin, mucosal surfaces, and connective tissues (Owen et al., 2018).

**Neutrophils** are the most abundant and short-lived innate immune cells that are among the first responders during acute inflammation. They detect and engulf pathogens into phagosomes, which fuse with granules containing antimicrobial peptides and generate reactive oxygen species to degrade ingested material. Neutrophils can also undergo degranulation, releasing proteolytic enzymes and inflammatory mediators, or form neutrophil extracellular traps (NETs) to immobilize and kill pathogens. After fulfilling their function, neutrophils undergo cell death processes, such as apoptosis, pyroptosis, ferroptosis, or NETosis. Dysregulated neutrophil activity contributes to infectious, metabolic, hereditary immune-mediated, and autoimmune diseases (Zhang et al., 2024).

#### 2.1.1.2 Monocytes and Macrophages

Monocytes circulate in the blood and migrate into tissues, where they can differentiate into macrophages. The tissue resident macrophages can originate at embryonic stage from progenitor cells and persist in close association with specialized tissue cells (Perdiguero et al., 2015). At the inflammatory sites, monocyte derived macrophages phagocytose pathogens, dead cells, and debris. They bridge innate and adaptive immunity by presenting antigens to lymphocytes and by secreting cytokines and growth factors that shape immune responses (Guan et al., 2025). Macrophages also contribute to tissue repair during chronic inflammation and fibrosis (Behmoaras et al., 2025). Depending on environmental cues, macrophages exhibit a continuum of activation states, with the classical M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes representing simplified extremes. Their functional states are implicated in a wide range of diseases, including cancer, autoimmune disorders, cardiovascular disease, and metabolic conditions (Guan et al., 2025).

#### 2.1.1.3 Dendritic Cells (DCs)

Dendritic cells are professional antigen-presenting cells that link innate and adaptive immunity. They patrol peripheral tissues, including the skin and mucosal surfaces,

for antigens. In the absence of inflammatory signals, they help maintain peripheral tolerance. DCs internalize antigens via phagocytosis, endocytosis, and other mechanisms, then migrate to lymph nodes where they present processed antigens via major histocompatibility complex (MHC) molecules to T cells, initiating T cell activation and differentiation (Anderson et al., 2021). Progenitors of DCs arise from bone marrow hematopoietic stem cells (HSCs) under tight control of transcriptional regulation. DCs can develop into multiple subsets (conventional cDC1 or cDC2 and plasmacytoid DCs) from a distinct developmental origin and exhibit specific functions. cDC1subsets present exogenous, cell associated antigens to CD8+ T cells and cDC2s present soluble antigens to CD4+ T cells. During viral infections, plasmacytoid DCs contributes to type 1 interferons production and recruit other immune cells (Anderson et al., 2021). During inflammation, monocytes can also differentiate into DCs (Bedford et al., 2020). The heterogeneity of DC subsets is critical for regulating diverse immune responses (Kanayama et al., 2025).

#### 2.1.1.4 Natural Killer (NK) cells and Innate Lymphoid Cells (ILCs)

NK cells and ILCs develop from common lymphoid progenitors (CLPs) but lack antigen-specific receptors and do not undergo clonal selection like adaptive lymphocytes. NK cells respond rapidly to infected or transformed cells. They express activating and inhibitory receptors that detect changes in MHC class I expression. Healthy cells expressing MHC class I deliver inhibitory signals that prevent NK-mediated killing, whereas virus-infected or tumor cells often downregulate MHC class I and upregulate activating ligands, triggering NK cell cytotoxicity. NK cells contain granules with perforin and granzymes that induce target-cell death and can also kill infected macrophages and dendritic cells. Depending on the tissue microenvironment, NK cells produce cytokines such as IFN-γ or TGF-β, contributing to inflammatory or immunosuppressive responses (Chen, Zhu, and Jounaidi, 2024; Elliott and Yokoyama, 2011).

ILCs are considered innate counterparts of CD4+ T helper cells. After migrating to peripheral tissues, they differentiate into ILC1, ILC2, or ILC3 subsets, mirroring the cytokine profiles and transcriptional programs of Th1, Th2, and Th17 cells. Unlike adaptive T cells, ILCs respond to cytokines, stress signals, and microbial cues rather than antigen recognition. They regulate local immune responses and can influence T cell activity directly through MHC class II or indirectly via dendritic cells (Eberl et al., 2015).

Although the innate immune system rapidly senses and eliminates microbes, the range of molecular patterns it can detect is limited. The vast diversity of pathogen structures and their ability to mutate to evade detection likely contributed to the evolutionary development of the adaptive immune system.

### 2.1.2 Adaptive immune system

The adaptive immune system relies on antigen-specific receptors generated through somatic recombination of a large array of gene segments. This process enables lymphocytes to recognize an enormous diversity of foreign antigens with high specificity. Because each receptor is unique to a particular antigen, the adaptive immune response is highly targeted.

Following an initial encounter with a pathogen, antigen-specific adaptive immune cells can persist for years or even a lifetime, forming the basis of immunological memory. Upon re-exposure to the same antigen, these memory cells are rapidly reactivated, expand in number, and mount an accelerated and more effective immune response (Lam, Lee and Farber, 2024).

Adaptive immune cells include T lymphocytes, which mature in the thymus, and antibody-producing B lymphocytes, which develop in the bone marrow. After maturation in these primary lymphoid organs, lymphocytes migrate to secondary lymphoid organs such as lymph nodes and the spleen. Here, they encounter antigens drained from peripheral tissues via lymph or circulating in the blood. Specialized antigen-presenting cells (APCs) of the innate immune system, such as DCs, also migrate to these secondary lymphoid sites to present processed antigens to adaptive immune cells. Once activated, lymphocytes disseminate throughout the body to exert their effector functions, providing either cellular or humoral adaptive immunity (Owen et al., 2018).

#### 2.1.2.1 T cells provide cellular adaptive immunity

T lymphocytes constitute the cellular arm of the adaptive immune system. Common lymphoid progenitors (CLPs) are derived from hematopoietic stem cells (HSCs) in the bone marrow and migrate to the thymus, where they undergo a series of developmental and lineage-commitment stages. Within the thymus, T cells that strongly recognize self-antigens are eliminated through negative selection, while those with T cell receptors (TCRs) capable of interacting appropriately with major histocompatibility complex (MHC) molecules are retained through positive selection (Ashby and Hogquist, 2024).

TCRs recognize specific peptide fragments of antigens presented on MHC molecules. The TCR complex consists of either an αβ heterodimer (expressed by the majority of T cells) or a γδ heterodimer (expressed by a minority of T cells). These heterodimers are non-covalently associated with invariant CD3 chains (CD3-γ, CD3-δ, CD3-ε, and CD3-ζ), which are essential for transmitting intracellular signals following antigen recognition (Notti et al., 2025).

When a TCR binds an antigen–MHC complex on an APC, it recruits co-receptors such as CD4 or CD8 to enhance signaling efficiency. Full T cell activation also

requires co-stimulatory signals, most notably through CD28. In the absence of co-stimulation, TCR engagement leads to T cell anergy, a state of functional unresponsiveness that prevents inappropriate or accidental immune activation. Co-stimulatory pathways ensure that T cell activation is context-appropriate and promote downstream signaling events that drive proliferation and differentiation. These pathways involve multiple protein kinases that regulate TCR-mediated signal transduction (Shah et al., 2021).

Based on the expression of CD4 or CD8 co-receptors, T cells are broadly classified into CD4+ and CD8+ subsets, each with distinct effector functions. CD4+ T cells recognize antigens presented on MHC class II molecules and primarily interact with professional APCs to coordinate and regulate immune responses. In contrast, CD8+ T cells recognize antigens presented on MHC class I molecules and function as cytotoxic T lymphocytes capable of directly killing infected or transformed target cells (Owen et al., 2018).

## 2.2 CD4+ T cells

CD4+ T cells play a central role in orchestrating immune responses against a wide range of pathogens while maintaining immune tolerance under healthy conditions. Through the secretion of cytokines, they coordinate the activity of other immune cells, including B cells for antibody production, CD8+ T cells for cytotoxic elimination of infected cells, and macrophages for pathogen clearance and activation of additional immune populations.

Naive CD4+ T cells become activated upon recognition of peptide–MHC class II complexes presented by antigen-presenting cells (APCs), together with essential co-stimulatory signals. The cytokine milieu present during activation is a critical determinant of their differentiation into distinct T helper (Th) subsets. Early studies identified Th1 and Th2 cells as two mutually exclusive helper lineages with distinct cytokine profiles and functional roles (Mosmann and Coffman, 1986). This binary model was later expanded with the discovery of IL-17 producing pro-inflammatory Th17 cells and forkhead box P3 (FOXP3) expressing, IL-2 dependent T regulatory cells (Tregs), revealing a broader and more complex landscape of CD4+ T cell specialization (Rogozynski and Dixon, 2024).

Advances in immunology have since identified additional Th subsets with unique surface receptors, lineage-defining transcription factors (TFs), and effector cytokines. CD4+ T cell subsets are now broadly classified into Th1, Th2, Th9, Th17, T follicular helper (Tfh) cells, and regulatory T cells (Tregs). Each subset is defined by specific priming cytokines, characteristic transcriptional programs, and distinct immunological functions (Figure 2). Collectively, these subsets contribute to host defense against diverse pathogens and play essential roles in immune regulation (Sun et al., 2023).

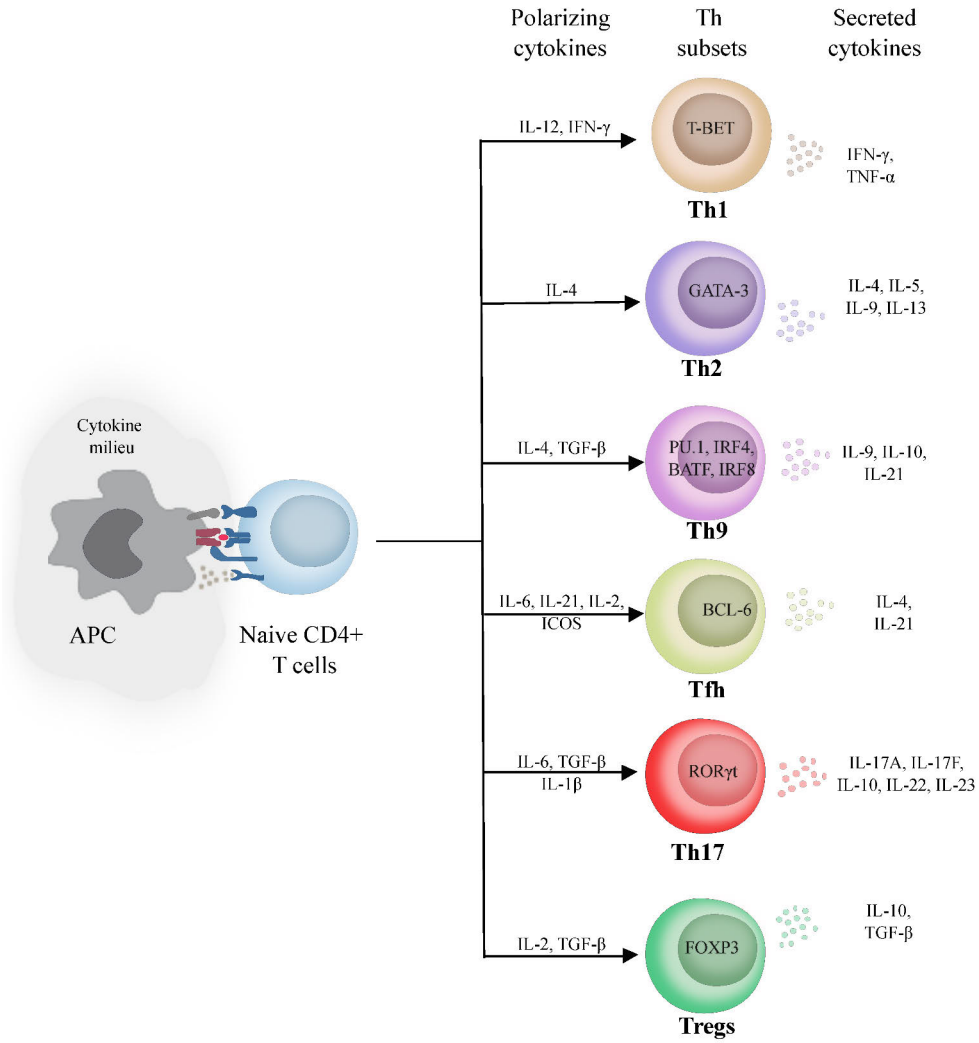


**Figure 2.** Naive CD4+ T cells upon activation by APC differentiate into different effector and regulatory T cells. The polarizing cytokine signaling regulates the initiation of distinct effector CD4+ Th cell subset lineage commitment along with TCR and co-stimulatory signaling. Modified from Sun et al., 2023.

## 2.2.1 Th1, Th2, Th9 and Tfh cells

### 2.2.1.1 Th1 cells

Th1 cells mediate immune response against intracellular pathogens such as viruses and certain bacteria. Their differentiation is driven by antigen presentation in the presence of IL-12, which activates STAT4 and induces T-bet (encoded by *Tbx21*),

the master regulator of Th1 lineage commitment (Thieu et al., 2008). T-bet promotes IFN-γ production, reinforcing Th1 differentiation and suppressing alternative Th2 and Th17 pathways. This initial signaling drives expression of Th1 associated genes and TFs that are crucial for maintaining Th1 phenotype. Th1 cells produce cytokines including IFN-γ, TNF-α, and IL-2. IFN-γ enhances macrophage activation, promote phagocytosis and cytotoxicity. TNF-α promotes inflammation and macrophage recruitment at infection site, and IL-2 supports the growth, differentiation and survival of cytotoxic immune cells critical for cellular immunity (Sun et al., 2023). Cells acquiring Th1 like phenotype and produce IFN-γ are implicated in the autoimmunity diseases (Sun et al., 2023).

#### 2.2.1.2 Th2 cells

Th2 cells are essential for immunity against extracellular parasites and play a central role in the pathogenesis of allergic diseases. Th2 differentiation occurs following antigen presentation in the presence of IL-4, which is often produced by mast cells and basophils at sites of infection. IL-4 activates STAT6 and induce expression of GATA3, a master regulator for Th2 lineage commitment. GATA3 promotes expression of Th2 cytokines (IL-4, IL-5, IL-13) and together with SATB1, facilitates chromatin remodeling at Th2 cytokine loci (Lee et al., 2000; Ahlfors et al., 2010). IL-4 drives B cell activation and IgE class switching, IL-5 enhances eosinophil responses, and IL-13 contributes to parasite clearance and tissue repair. Dysregulated Th2 responses drives allergic asthma, chronic rhinosinusitis, and atopic diseases (Matthias and Zielinski, 2019).

#### 2.2.1.3 Th9 cells

Th9 cells are a more recently identified CD4+ T cell subset characterized by high IL-9 production. They play important roles in immunity against helminths and contribute to allergic and asthmatic inflammation (Park et al., 2022). Th9 differentiation is initiated by antigen presentation in the presence of IL-4 and TGF-β (Dardalhon et al., 2008; Veldhoen et al., 2008). IL4 activates STAT6 and induces GATA3, IRF4 and BATF, which promote IL-9 transcription, while TGF-β activates SMADs, PU.1 and IRF8 supporting further differentiation. IRF4 and PU.1 are identified as a lineage defining TF essential for IL-9 expression. Although initially thought to be a branch of Th2 cells, these cells are now recognized as a distinct subset with unique functions as well as some overlapping function with Th2 cells (Kaplan et al., 2015).

#### 2.2.1.4 Tfh cells

Tfh cells are specialized CD4+ T cells that regulate germinal center formation and function within secondary lymphoid organs. They provide critical help to B cells to enable the generation of high-affinity antibodies, long-lived plasma cells, and memory B cells. IL-6 signaling induces BCL6, the master regulator of Tfh differentiation, which drives expression of Tfh markers such as CXCR5, ICOS, and PD-1 (Crotty et al., 2014; Yu et al., 2009).

### 2.2.2 Tregs

Regulatory T cells (Tregs) are essential for the maintenance of peripheral immune tolerance and prevent chronic inflammatory and autoimmune diseases by suppressing the activity of other immune cells. Tregs are recruited within the tumor microenvironment, where they contribute to immunosuppression and support tumor progression (Liu, Workman, and Vignali, 2016).

A distinct population of CD4+ T cells expressing high level of IL-2 receptor α-chain (IL-2Rα/CD25) was first identified to be critical for self-tolerance to maintain immune homeostasis. The depletion of these CD25+ CD4+ T cells resulted in down-regulation of suppressive immune response against antigens of self-origin when compared with non-self-origin and contributed to autoimmune pathology (Sakaguchi et al., 1995). This was later supported by findings in scurfy mice and in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, where mutations in *FOXP3* disrupt immune homeostasis (Bennett et al., 2001; Brunkow et al., 2001). Forced expression of FOXP3 in CD4+ T cells confer suppressive capacity, whereas FOXP3 deficiency abrogates Tregs function. FOXP3 was subsequently established as the lineage-defining TF for Tregs (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003).

Tregs can be broadly classified based on their developmental origin. Thymic Tregs (tTregs), also known as natural Tregs (nTregs) develop in the thymus. Peripheral Tregs (pTregs) arise from conventional CD4+ T cells in peripheral tissues such as the intestinal lamina propria. Similar to pTreg, conventional or naive CD4+ T cells can be stimulated *in vitro* to differentiate into FOXP3+ T cells called induced Tregs (iTregs) (Wing et al., 2019).

Tregs differentiation requires TCR engagement, CD28 co-stimulation, and cytokines such as TGF-β and IL-2. These signals activate transcriptional and epigenetic pathways that induce FOXP3 expression (Lee and Lee, 2018). NF-κB, AP-1, and NFAT bind *FOXP3* regulatory regions following TCR/CD28 signaling, while TGF-β promotes *FOXP3* transcription through SMAD2/3 and FOXO1/3. IL-2–STAT5 signaling further enhances FOXP3 expression by binding its conserved non-coding sequences (CNS). FOXP3 also maintains its own expression through an

autoregulatory loop and controls a core transcriptional program essential for Tregs stability and suppressive function (Sun et al., 2023). Recent findings suggest, iTregs generated from conventional or effector/memory CD4+ T cells with deprivation of CD28 co-stimulation signaling induce functionally stable Tregs with high FOXP3 (Mikami et al., 2020; 2025).

Tregs suppress immune responses through multiple mechanisms, including expression of cell contact inhibitory receptors (CTLA-4, LAG-3, TIGIT, CD73, CD39), secretion of immunosuppressive cytokines (IL-10, TGF-β, IL-35) and metabolic control (Wang et al., 2025). Tregs exhibit substantial plasticity and can adopt Th-like phenotypes by expressing lineage-specific TFs and chemokine receptors, enabling them to migrate to inflamed tissues and suppress corresponding effector Th subsets (Trujillo-Ochoa, Kazemian, and Afzali, 2023). Although Tregs numbers vary across autoimmune diseases, impaired Tregs function is a consistent feature (Sun et al., 2023).

In cancer, tumor infiltration of Tregs and their relative abundance compared to conventional T cells generally correlate with prognosis. Abundance of Tregs within tumor is largely responsible for limiting tumor clearance by CD4+ and CD8+ T effector cells. Tregs are associated with poor prognosis in certain cancer types including melanoma, breast, non-small-cell lung, cervical, gastric, renal, bladder, endometrial, and ovarian cancers. However, in certain malignancies—including Hodgkin's lymphoma, HNSCC, colorectal cancer, esophageal cancer, and oral/oropharyngeal squamous cell carcinoma—Tregs infiltration is associated with improved outcomes (Sun et al., 2023). TME-driven Tregs plasticity and acquisition of Th-like phenotypes may contribute to these contrasting roles by shifting the balance between immunosuppressive and pro-inflammatory functions (Imianowski et al., 2025; Togashi et al., 2019).

## 2.3 Th17

Th17 cells are a distinct lineage of CD4+ T cells characterized by high production of pro-inflammatory IL-17 family cytokines, primarily IL17A (commonly referred to as IL-17) and IL17F (Figure 2). IL-17 acts on innate immune cells and epithelial cells to induce granulocyte colony-stimulating factor (G-CSF) and IL-8 (CXCL8), which promote neutrophil production and recruitment to the site of infection for pathogen clearance (Isailovic et al., 2015). Through these mechanisms, Th17 cells contribute to pathogen clearance, particularly against extracellular bacteria and fungi.

However, Th17 cells exhibit functional duality. Depending on the antigenic stimulus and cytokine milieu, they can mediate protective immunity or drive

immunopathology. This functional divergence has led to the classification of Th17 cells into pathogenic and non-pathogenic subsets.

### 2.3.1 Pathogenic and non-pathogenic Th17 cells

The identification of Th17 cells emerged from studies of autoimmune disease models. Earlier paradigms attributed autoimmune pathology primarily to Th1 cells, driven by IL-12 and producing IFN-γ. However, work in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), challenged this view. Mice deficient in IFN-γ or its receptor still developed severe EAE with infiltration of activated CD4+ T cells (Krakowski and Owens, 1996; Tran et al., 2000), indicating that Th1 cells were not the sole drivers of disease.

The discovery of IL-23, a heterodimer composed of p40 and p19 subunits that is distinct from structurally related IL-12 (p40/p35), further shifted this understanding (Oppmann et al., 2000). Earlier studies targeting the shared p40 subunit inadvertently disrupted both IL-12 and IL-23, confounding interpretations of Th1 involvement. IL-23 was shown to promote an activation state of CD4+ T cells that is distinct from Th1 and Th2 cells (Aggrawal et al., 2003). Subsequent work using *Il-23*- or *Il-12*-specific knockout mice demonstrated that Il-23, not Il-12, is essential for autoimmune inflammation (Cua et al., 2003; Murphy et al., 2003). Three consecutive studies established Il-23–responsive, IL-17–producing CD4+ T cells as a distinct pathogenic lineage (Harrington et al., 2005; Langrish et al., 2005; Park et al., 2005).

While pathogenic Th17 cells contribute to autoimmune disease, non-pathogenic Th17 cells play critical roles in the maintenance of mucosal homeostasis and defense against microbial invasion (Figure 3). Infante-Duarte et al. showed that *Borrelia burgdorferi* primed murine naive CD4+ T cells to produce IL-17, TNF-α, and GM-CSF, distinct from Th1/Th2 profiles. Similar IL-17 responses were observed in synovial fluid from Lyme arthritis patients (Infante-Duarte et al., 2000).

Th17 cells also mediate protective homeostatic immunity against *Klebsiella pneumoniae* lung infection (Happel et al., 2005), maintain intestinal barrier integrity through RORγt$^{+}$ Th17 cells in the lamina propria, and promote epithelial tight-junction formation (Kinugasa et al., 2000). Neutralization of IL-17 exacerbated colitis in mouse models, highlighting its protective role in epithelial repair (Ogawa et al., 2004; Ivanov et al., 2006). Th17 cells are also essential for immunity against *Candida albicans* (Saijo et al., 2010) and are induced by *S. aureus* in human monocyte–T cell co-cultures (Zielinski et al., 2012). Collectively, these studies established IL-17–producing Th17 cells as key mediators of non-pathogenic host defense (Mills, 2023).

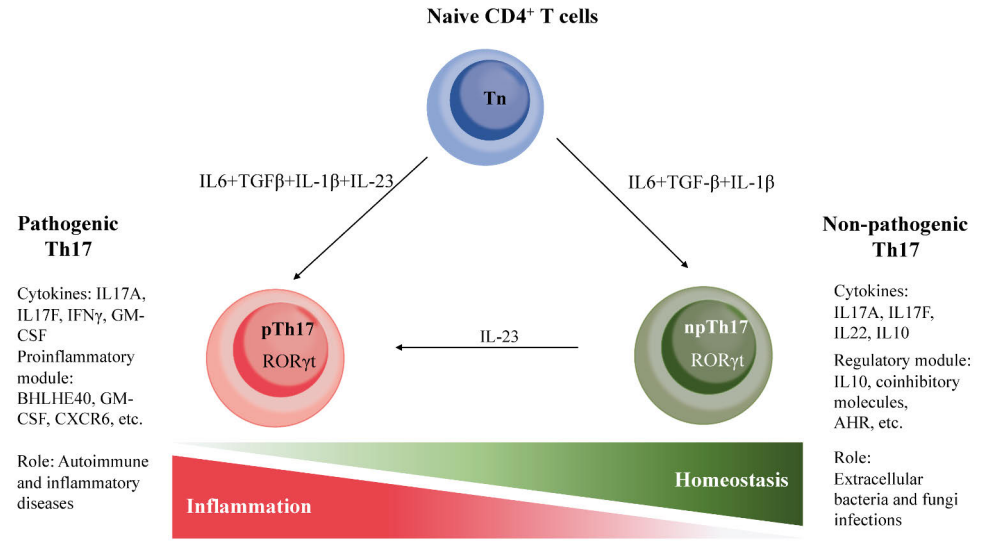


**Figure 3.** Th17 cells exhibit functional heterogeneity. The non-pathogenic (npTh17) cells are important in the immune homeostasis while pathogenic (pTh17) cells contribute to their effector function of tissue inflammation. IL-23 and IL-1β induce a common proinflammatory module including BHLHE40, GM-CSF, IFN-γ and CXCR6 By contrast, IL-27 induces the expression of a common regulatory module that includes anti-inflammatory molecules such as IL-10 and coinhibitory molecules. Modified from Schnell, Littman and Kuchroo, 2023.

## 2.3.2 Differentiation of Th17 cells

Th17 differentiation is regulated by a complex interplay of cytokines and pioneering transcription factors (Figure 4). Early studies revealed species-specific differences between mouse and human Th17 induction; however, later work using human cord-blood naive T cells and serum-free culture conditions demonstrated that the core molecular requirements are conserved (Korn et al., 2009).

### 2.3.2.1 Cytokine requirements

In mice, the induction of Th17 differentiation is initiated by antigen presentation together with IL-6 and TGF-β (Bettelli et al., 2006; Veldhoen et al., 2006). However, in the absence of IL-6, differentiation of Th17 can be induced by IL-21 along with TGF-β (Yang et al., 2008). IL-21, in the presence of TGF-β amplifies Th17 responses through a positive feedback loop (Korn et al., 2007). IL-23 is not required for initial differentiation but is essential for stabilizing and promoting the pathogenic Th17 phenotype (McGeachy et al., 2007). In humans, IL-1β plays an additional critical role alongside IL-6 in initiating Th17 differentiation (Figure 4; Acosta-Rodriguez et al., 2007; Ghoreschi et al., 2010; Korn et al., 2009; Wilson et al., 2007).

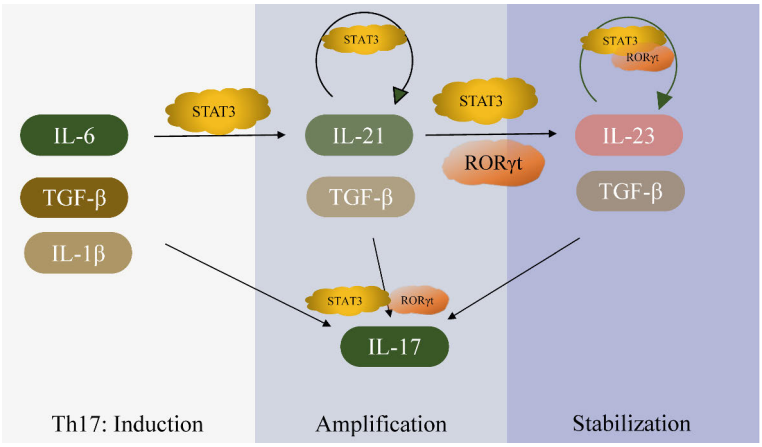


**Figure 4.** IL-6, TGF-β, IL-1β, IL-21 and IL-23 cytokine signaling are essential for *in vitro* differentiation of human Th17 cells with the pathogenic phenotype whereas IL-23 is not required for the non-pathogenic Th17 cells. Modified from Ivanov, Zhou and Littman, 2007.

#### 2.3.2.2 Transcription factor requirements

TGF-β activates SMAD signaling, while IL-6 activates STAT3 and both are essential for inducing retinoic acid receptor-related orphan receptor-γt (RORγt), the master regulator of Th17 differentiation (Ivanov et al., 2006; Korn et al., 2009). RORγt upregulates Th17-associated cytokines (IL-17A, IL-17F, IL-21, IL-22) and coordinates expression of additional TFs required for Th17 stability.

STAT3 enhances IL-23R expression, promoting terminal pathogenic differentiation, and induces HIF-1α, which suppresses FOXP3 and favors Th17 development. IL-1β activates IRF4, Akt/mTOR, and MAPK pathways, further supporting Th17 differentiation and IL-21 production. TGF-β suppresses Th1- and Th2-associated transcription factors (T-BET and GATA3), thereby facilitating Th17 polarization (Figure 5; Muranski and Restifo, 2013).

#### 2.3.2.3 Balancing Th17 and Tregs differentiation

TGF-β is required for both Th17 and Tregs differentiation. High concentrations favor $FOXP3^+$ Tregs induction, while lower concentrations promote Th17 differentiation. FOXP3 and RORγt interact with RUNX1 and reciprocally regulate each other (Zhang et al., 2008). IL-6–STAT3–HIF-1α signaling inhibits FOXP3 induction, shifting differentiation toward Th17 cells (Figure 5).

Non-pathogenic Th17 cells produce IL-10, contributing to mucosal homeostasis and limiting inflammation. IL-10 can also promote Th17 plasticity toward a Tregs-like phenotype. The Th17/Tregs balance is controlled by transcriptional and epigenetic mechanisms, including chromatin remodeling at key regulatory loci (Muranski and Restifo, 2013).

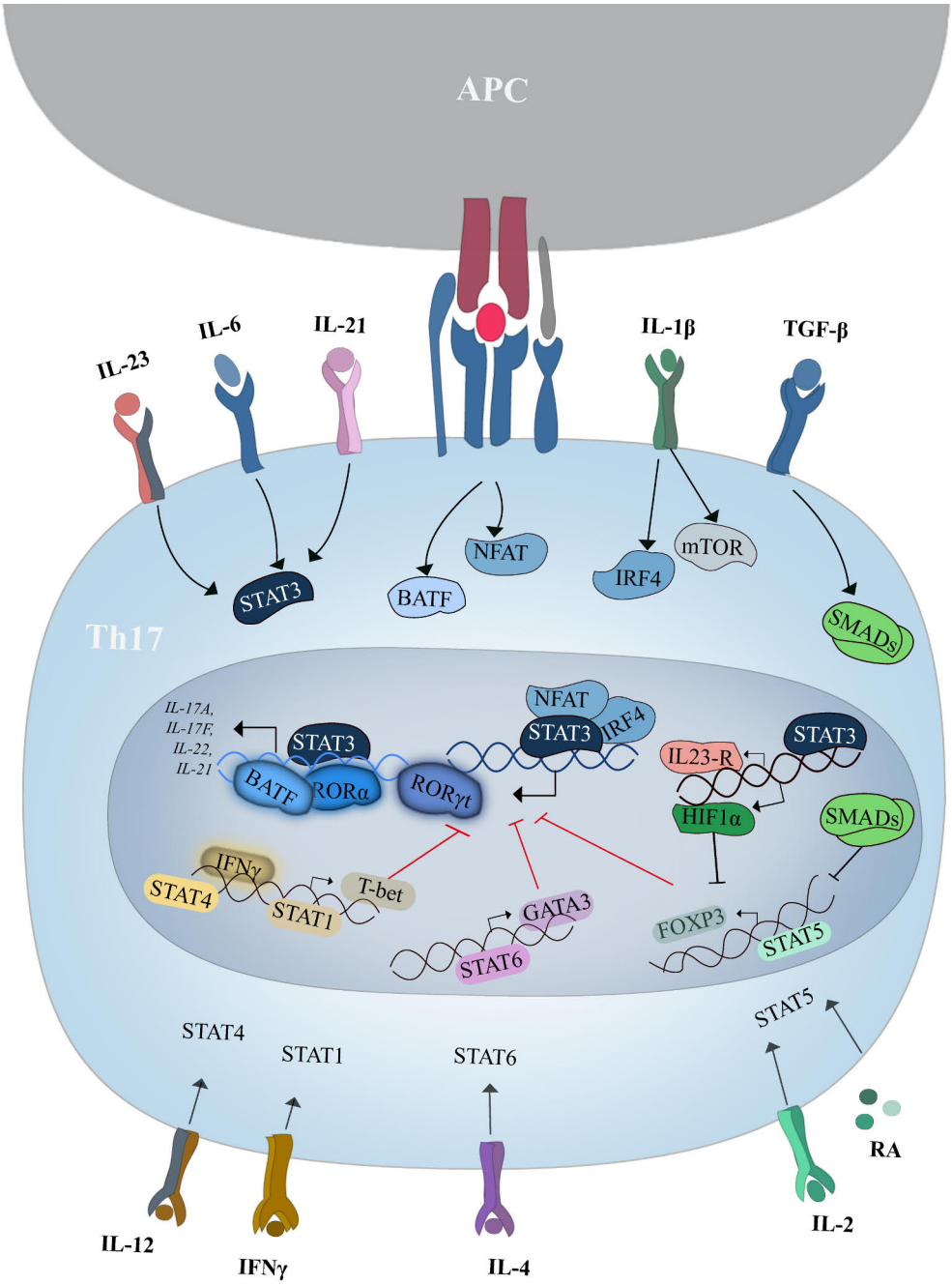


**Figure 5.** TCR and cytokine signaling induce Th17 differentiation where various transcription factors coordinate Th17 cytokine expression including IL17A, IL17F, IL21 and IL22. Key Th17 associated TFs inhibit other Th1, Th2 and Tregs lineages. Modified from Muranski and Restifo, 2013.

### 2.3.3 Transcriptional regulation of Th17

RORγt was the first lineage-defining transcription factor (TF) identified for Th17 cells (Ivanov et al., 2006). Although essential, RORγt alone is insufficient to establish the full Th17 transcriptional program. RORα, a related nuclear receptor, can partially compensate for RORγt deficiency and regulates an overlapping set of Th17-associated genes (Castro et al., 2017; Yang et al., 2008).

STAT3 is critical for IL-17 signaling, a role highlighted by human genetic disorders such as autosomal dominant hyper-IgE syndrome. Mutations in STAT3 impair IL-17 producing Th17 cells and lead to recurrent infections with *Candida*, *Staphylococcus*, and *Aspergillus* species (Holland et al., 2007; Milner et al., 2008; Minegishi et al., 2007). Studies in both humans and mice show that STAT3 directly binds to key Th17-related TFs including RORγt, BATF, and IRF4 as well as cytokines and cytokine-receptor genes such as *IL17a*, *IL17f*, and *IL23R*, underscoring its central role in Th17 differentiation (Durant et al., 2010; Tripathi et al., 2017).

Activating protein 1 (AP-1) super family transcription factors such as JUN, FOS, MAF and ATF family proteins forms homo- or heterodimers and mediate transcriptional regulation during T helper cell differentiation (Katagiri et al., 2021). JUN proteins homodimers or heterodimers with FOS proteins regulate Th17 differentiation (Carr et al., 2017; Moon et al., 2017). BATF, a member of the AP-1 transcription factor family, is activated downstream of TCR signaling and forms heterodimers with other AP-1 proteins such as JUNB to regulate Th17 differentiation (Schraml et al., 2009). Recent work (Study II) demonstrated that BATF competes with FOSL1 and FOSL2 at key Th17 gene loci, thereby regulating their expression during Th17 differentiation (Shetty et al., 2022).

IRF4 is another essential TF required for Th17 development as well as Th2, Th9 and Tregs lineages (Brüstle et al., 2007; Cretney et al., 2011; Lohoff et al., 2002; Staudt et al., 2010). BATF promotes IRF4 expression, and together these factors form a cooperative complex that regulates Th17 specific target genes (Li et al., 2012). A crucial role of IRF4 in Th17 development is demonstrated by *irf4* depletion in mice which showed reduced Th17 cell numbers and resistance for EAE development (Brüstle et al., 2007).

Thus, taken together, full Th17 differentiation requires coordinated activity of multiple transcription factors, including STAT3, IRF4, BATF, HIF-1α, and RUNX1.

Using newly emerged genome wide sequencing approaches, two landmark studies mapped the transcriptional regulatory network governing murine Th17 differentiation. Various TFs were identified to play role in Th17 differentiation (Ciofani et al., 2012; Yosef et al., 2013). Ciofani *et al.,* used ChIP-seq and TF-mutant profiling to identify co-occupancy of STAT3, IRF4, BATF, c-MAF, RORγ, and p300 at the key Th17 loci such as *Il17a, Il17f, Il12rb1, Il1r1* and *Rorc*. BATF and

IRF4 acted as pioneering factors establishing chromatin accessibility and enabling STAT3-driven transcriptional activation. Subsequently, RORγt along with other TFs refined important Th17 gene loci and stabilized the Th17 program. c-Maf targeted an anti-inflammatory gene repertoire and globally repressed proinflammatory genes that are regulated by other core TFs. FOSL2, an AP-1 family TF was identified as a negative regulator of Th17 differentiation and plasticity under inflammatory conditions. FOSL2 formed highly interconnected network with core TFs. FOSL2 repressed BATF expression and showed high degree of overlap with BATF binding sites (Ciofani M et al., 2012).

Yosef *et al.,* performed time-series transcriptional profiling across early (induction), intermediate (amplification), and late (stabilization) phases. The early phase induction was marked with known initiator factors such as STAT3, BATF and IRF4 while intermediate was expressing RORγt, AHR and other TFs. They identified 71 regulatory factors, validated 39 of them through gene perturbation, and classified them into positive and negative regulatory modules. Perturbations affected expression of other Th-lineage signature genes, highlighting the interconnectedness of Th17 and other Th subsets. Mina, Fas and Pou2af1 were shown to promote Th17 by suppressing FOXP3 program, IFN-γ and IL-2 expression, respectively. Studies on Tsc22d3 knockdown suggested it may limit Th17 in a negative feedback loop and be important in inflammation functions (Yosef et al., 2013).

Over the past decade, transcriptomic studies have identified numerous genes involved in Th17 differentiation (Acerbi et al., 2016; Buchacher et al., 2023; Capone et al., 2021; Gaublomme et al., 2015; Hu et al., 2017; Thakore et al., 2024). However, several limitations remain: *In vitro* culture systems often fail to fully recapitulate physiological differentiation conditions and may not reflect the heterogeneity of Th17 cells (Gaffen et al., 2014; Stockinger and Omenetti, 2017). Early human studies were confounded by differences in culture media and the use of non-naive T cells. Many *in vivo* studies rely on mouse models such as EAE, which emphasize pathogenic Th17 cells, whereas non-pathogenic Th17 cells and early differentiation events are equally important for understanding their lineage specification. Comparative transcriptomics revealed substantial differences between mouse and human Th17 cells, with only core regulatory elements conserved (Tuomela et al., 2016). Therefore, validation of novel Th17-associated genes in human Th17 cells is essential before extrapolating findings from animal models to human clinical trials (Stockinger and Omenetti, 2017). Emerging single-cell technologies will be crucial for resolving Th17 heterogeneity and enabling more accurate interpretation of lineage-specific transcriptional programs.

### 2.3.4 Epigenetic regulation of Th17 differentiation

Epigenetic mechanisms are central to the remarkable plasticity of Th17 cells and their ability to adopt features of other T cell lineages. Epigenetic modifications influence chromatin structure and thereby regulate TFs access to lineage-defining loci at T cell subsets (Wei et al., 2009). Histone methylation at specific lysine residues of histone 3 is associated with active or repressed transcription of important genes. Histone acetylation, particularly H3K27ac, neutralizes the positive charge of histones, loosening DNA–histone interactions and promoting chromatin accessibility. Histone deacetylase (HDACs) enzymes remove acetyl groups, restoring chromatin compaction and limiting transcription factor binding (Renaude et al., 2020). DNA methyl transferase (DNMTs) enzymes facilitate addition of methyl group at the promoter CpG islands that typically represses gene expression. Post-transcriptional regulation by non-coding RNAs, including microRNAs and lincRNAs, further modulates gene expression by destabilizing mRNA or inhibiting translation (Renaude et al., 2020).

Active promoters are marked by H3K4me3 and H3K27ac, whereas enhancers typically carry H3K4me1 together with H3K27ac. In contrast, inactive promoters are enriched for H3K27me3, and inactive or repressed enhancers exhibit H3K4me1 without acetylation or bear repressive marks such as H3K9me3 or H3K27me3 (Renaude et al., 2020). Epigenetic modifications such as H3K4me3 and H3K27ac are essential for the expression of key Th17 genes, including RORC and IL17, while simultaneously repressing lineage-defining genes of other T-cell subsets (Akimzhanov et al., 2007; Mukasa et al., 2010). Th17 cells often retain poised epigenetic states at loci such as GATA3, TBX21, and FOXP3, reflecting their inherent plasticity (Wei et al., 2009). CXXC finger protein 1 maintains H3K4me3 at the IL-6Rα, IRF4, BATF, and other transcription factor loci, thereby supporting the epigenetic programs required for sustained Th17 differentiation (Lin et al., 2019).
Clusters of enhancers located in close genomic proximity can form super-enhancers (SEs), which recruit high densities of transcriptional activators and regulate lineage-defining genes (Hnisz et al., 2013). Mapping the enhancer landscape in mouse Th17 cells using p300 ChIP-seq and transcription factor binding profiles has revealed extensive enhancer remodeling during differentiation (Vahedi et al., 2015). TRIM28 binding is enriched at SEs, including those at the IL17 and IL17F loci in Th17 cells (Jiang et al., 2018). Assay for transposase-accessible chromatin using sequencing (ATAC-seq) has further identified regulatory networks that control Th17-specific gene expression. A proximal enhancer of *Rorc* was shown to promote its expression in mouse Th17 cells (Tian et al., 2021). Similarly, study III combined histone ChIP-seq and ATAC-seq profiling during human Th17 differentiation, and demonstrated that Th17-specific SEs strongly correlate with increased expression of

nearby genes. An intronic SE within RORA was found to regulate both RORA and IL17 expression (Kalim et al., 2024).

Post-translational modifications of transcription factors also contribute to Th17 differentiation. The HAT p300 acetylates RORγt within its DNA-binding domain, enhancing its transcriptional activity, whereas HDAC1 reverses this modification. SIRT1 deacetylates STAT3, reducing its nuclear translocation and impairing Th17 differentiation (Limagne et al., 2017). SUMOylation further enhances RORγt-dependent transcription. Additionally, several E3 ubiquitin ligases including TRAF5, USP4, USP15, USP17, UBR5, and DUBA regulate RORγt stability and thereby influence Th17 cell fate (Zhang et al., 2021).

### 2.3.5 Th17 cells contribute to autoimmune diseases

Th17 cells contribute to both protective immunity and immune-mediated pathology. Dysregulation of the Th17/Tregs balance is implicated in autoimmune diseases, chronic inflammation, and cancer.

In rheumatoid arthritis (RA), Th17 cells infiltrate synovial tissue and joint fluid. The increase in the proportion of Th17 cells and the decrease in the proportion of Tregs cells in the peripheral blood of RA patients indicates the Th17/Tregs imbalance and correlates with disease severity (Paradowska-Gorycka et al.,2020). Th17 cells in RA can acquire a Th1-like phenotype (Th1-like ex-Th17) by producing IFN-γ, contributing to pathology and resisting Tregs-mediated suppression (Basdeo et al., 2017, Mills et al., 2023). Similar IFN-$\gamma^+$ Th17 populations are observed in MS and colitis. Th17/Tregs imbalance is also evident in systemic lupus erythematosus (SLE) (Park and Ciofani, 2025). In Graves' disease, elevated IL-17A, IL-6, IL-23, and IL-1β levels are observed in orbital tissues of patients with thyroid-associated ophthalmopathy (Fang et al., 2019). In psoriasis, the inflamed skin lesions contain IL23 producing Th17 cells (Park and Ciofani, 2025).

## 2.4 Type 1 diabetes

Type 1 diabetes is a chronic autoimmune disease where insulin secreting pancreatic islet β-cells are progressively destroyed by various mediators of the immune system. Under normal physiological conditions, upon food intake, β-cells sense rising blood glucose via its uptake through glucose transporters and subsequent metabolism by glucokinase, leading to an increase in the ATP/ADP ratio, closure of ATP-sensitive $K^+$ channels, membrane depolarization, $Ca^{2+}$ influx, and release insulin (Roep et al., 2021). The released insulin maintains stable metabolism by promoting uptake of carbohydrates, proteins and lipids into cells. Insulin enhances peripheral glucose uptake in muscle and adipose tissue and rapidly inhibits hepatic

gluconeogenesis and glycogenolysis, thereby lowering blood glucose levels. This reduction in glucose subsequently downregulates insulin secretion by β-cells (Mathieu et al., 2017).

In T1D, immune-mediated destruction of β-cells leads to insufficient or no insulin that affects glucose metabolism, resulting in hyperglycemia, increased protein catabolism, lipolysis and formation of ketone bodies (Mathieu et al., 2017). Thus, in patients not treated with exogenous insulin, dehydration by hyperglycemia and ketone accumulation causes diabetic ketoacidosis, a life-threatening condition.

Before the discovery of insulin in 1921, T1D was universally fatal. The introduction of exogenous insulin therapy (Banting et al., 1922) transformed T1D into a manageable chronic disease. Despite major advances in understanding disease mechanisms over the past century, insulin replacement remains the only effective treatment and requires continuous glucose monitoring to maintain metabolic control. There are major challenges with insulin treatment, mainly to mimic the action profile of physiological β-cell insulin which includes a constant basal level and rapid peaks during food intake. Since insulin is peripherally administered, there are no feedback mechanisms to suppress its levels when glucose levels fall and thus increase risk of hypoglycemia (Mathieu et al., 2017). T1D is associated with reduced quality of life, serious-long term complication, decreased life expectancy and substantial economic burdens for individuals and health care systems (Gregory et al., 2022).

In 2024, an estimated 9 million people worldwide were living with T1D and approximately half a million new cases were diagnosed each year. Notably, among the diagnosed cases, 18% individuals were younger than 20 years of age (Bell and Lane, 2025). Although long regarded as a juvenile autoimmune disease, T1D can develop at any age. The incidence of T1D continues to rise globally, particularly among young children (Gregory et al., 2022; Stene, 2024). The frequency of children presenting with diabetic ketoacidosis (DKA) at diagnosis has also increased. Finland reports the highest incidence of T1D in children under 15 years, with approximately 52 cases per 100,000 annually between 2015 and 2018. Rising incidence is attributed to interactions between genetic susceptibility, environmental triggers, and lifestyle changes (Knip, 2021).

### 2.4.1 Pathogenesis of T1D

T1D is a multifactorial disease driven by both genetic predisposition and various environmental triggers that initiate autoimmune activation. Immune cells recognize self-antigens derived from pancreatic β-cells. The appearance of autoantibodies (Aab) to self-antigens is the earliest indication of onset of islet autoimmunity and ongoing disease pathogenesis (Kallionpää et al., 2019). The Aab includes insulin Aab (IAA), glutamic acid decarboxylase Aab (GADA),

insulinoma associated antigen 2 Aab (IA2) and zinc transporter (ZnT8) Aab. Because Aabs can be detected in blood plasma, they serve as important biomarkers for identifying individuals at the risk of disease progression (Insel et al., 2015). The rate of disease progression from islet autoimmunity to clinical diagnosis varies widely, ranging from months to several years before appearance of glucose abnormality and disease symptoms. Aab are often detected at young age in children and adolescents while it may take until adulthood before the disease will be manifested or diagnosed (Jia and Yu, 2024).

More than 40 years ago, George Eisenbarth proposed a model in which genetically susceptible individuals exposed to environmental triggers develop progressive autoimmune destruction of β-cells, leading to a linear decline in β-cell mass (Eisenbarth, 1986). This framework has since evolved into a staging model (Figure 6) by Insel *et al.,* that categorizes T1D into pre-symptomatic and symptomatic phases based on measurable autoimmune markers (gene susceptibility and Aabs), glycemic abnormalities, and other clinical symptoms (Insel et al., 2015).

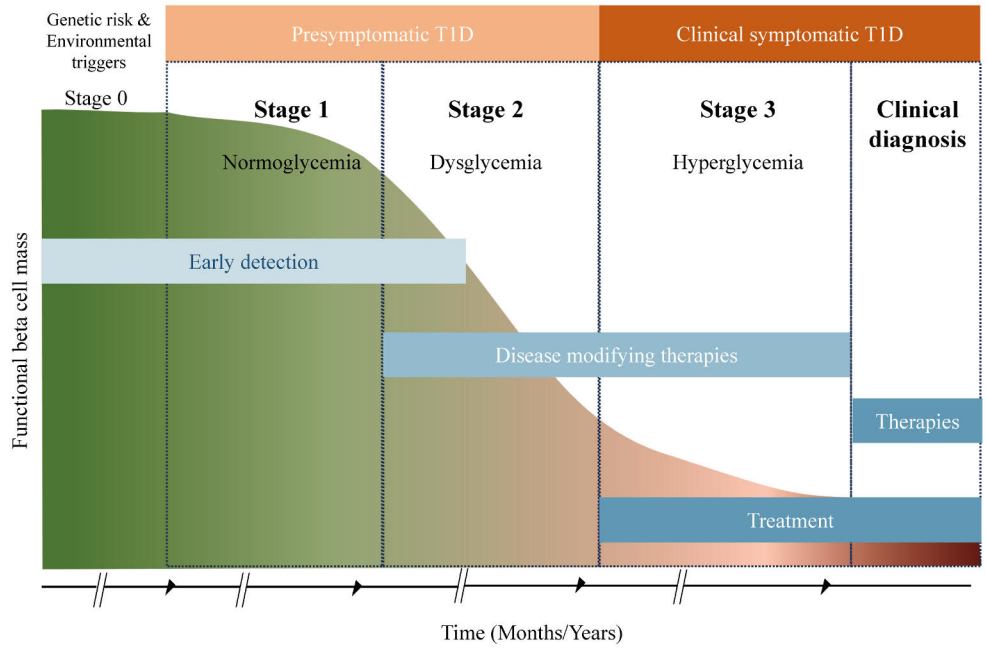


**Figure 6.** Staging model of T1D progression. Modified from Insel et al., 2015.

## Staging of T1D

Stage 1 represents individuals who have developed two or more T1D associated Aab but normal glucose tolerance (normoglycemic). Stage 2 includes two or more islet

T1D associated Aab and the disease has progressed to show dysglycemic symptoms due to loss of functional β-cell mass. Stage 3 represents manifestation of typical clinical symptoms of hyperglycemia due to considerable loss of insulin producing β-cells (Figure 6). The symptoms may include polyurea, polydipsia, weight loss, fatigue, diabetic ketoacidosis (DKA) and others (Insel et al., 2015). Recent work suggests further subclassification of stage 3 into stages 3a, 3b, and 3c based on residual insulin secretion and clinical characteristics (Tatovic, Narendran and Dayan, 2023).

This staging model (Figure 6) has greatly facilitated screening and identification of individuals at risk, particularly those with genetic susceptibility. The 5–6-year risk of clinical diagnosis for individuals with stage 1 is 35–50%, for individuals at stage 2 it is 75% whereas all stage 3 individuals are predicted to develop the clinical disease during their lifetime (Herold et al., 2024). The model assumes a generally linear progression through three stages and enables population-level risk stratification, early monitoring, and timely intervention to reduce the likelihood of life-threatening DKA. The staging model has aided various clinical trials, including on teplizumab, that is aimed at delaying the progression of disease in individuals that are at high-risk stages like stage 2.

However, there are many limitations to this model due to heterogeneity of disease progression in different individuals which is influenced by factors like age, environmental exposure like infections and many others. More precise tools are needed to identify individuals at highest risk, particularly for immunotherapy-based disease modifying therapies. Reframing of this model with a T1D risk determination calculator is proposed to be beneficial for prevention and delaying of the disease onset (Ahmad et al., 2023).

In the staging model (Figure 6), the emergence of Aab is preceded by an asymptomatic period during which functional beta cell mass is slowly decreased. This pre-stage 1 is also referred as stage 0 in different studies and is characterized by genetic predisposition and associated risk of autoimmunity due to potential environment triggers (Michels et al., 2025). This stage is particularly of interest for an early identification of mechanisms involved in the initiation of disease. This time window is critical for early diagnosis of disease before considerable autoimmune damage is detected at the seroconversion stage. The timely intervention in this stage can avert the onset of disease in clinical trials before reaching to stages of disease progression.

### 2.4.2 Genetic predispositions and environmental triggers in T1D

Early twin studies demonstrated strong genetic contributions to T1D, with monozygotic twins showing much higher concordance than dizygotic twins.

Although familial clustering is evident, approximately 90% of individuals diagnosed with T1D do not have a family history. The children born in a family with an affected family member have a 5% risk of developing T1D, compared with a 0.3–0.6% for children without affected family members. In the sibling, about 8% risk noted which can exceed 70% for the twins (Bonifacio et al., 2010; Herold et al., 2024; Turtinen et al., 2019).

The human leukocyte antigen (HLA) provides highest genetic risk for T1D that accounts for 40-50% of familial predisposition. The haplotypes of HLA associated with T1D are DR3-DQ2 and DR4-DQ8 observed in almost 90% individuals with disease (Turtinen et al., 2019). Since HLA genes affect the diversity and specificity of immune responses, immune mediated pathogenesis in strongly suggested to initiate the disease.

The genome-wide association studies have revealed that T1D is a polygenic disease with more than 143 loci involving over 60 genes that contribute to the overall genetic risk for disease (Robertson et al., 2021). Many of these genes influence β-cells function or regulate T cell function, activation and differentiation. Some of the key genes are *PTPN22*, *CTLA4* and *IL2RA*, the latter two being essential for Tregs function. Defects in Tregs activity have been implicated in T1D pathogenesis both in humans and in mouse models (Herold et al., 2024).

Environmental triggers also play a significant role. Viral infections, dietary exposures and microbiome alterations have been widely studied in a number of observational studies (Herold et al., 2024). Early histopathological analyses detected viral capsid proteins in pancreatic islets of individuals with T1D. Modern sequencing approaches have strengthened the association between enterovirus infection particularly Coxsackie B virus and the development of islet autoantibodies and progression to clinical disease (Nekoua, Alidjinou, and Hober, 2022).

The dysregulated microbiome, possibly linked to changes in gut permeability or viral infections has been reported in T1D (Del Chierico et al., 2022). Microbiome-driven changes in innate immune populations, such as mucosal-associated invariant T (MAIT) cells and innate lymphoid cells (ILCs), which help maintain gut-barrier integrity, may contribute to disease pathogenesis (Rouxel et al., 2017).

### 2.4.3 Role of innate immune cells in T1D

Innate immune cells are increasingly recognized as important contributors to the initiation and progression of T1D (Figure 7). Studies using the non-obese diabetic (NOD) mouse model have demonstrated that several innate immune cell types influence pancreatic β-cell destruction. Although the precise roles of these cells in humans are still being clarified, available evidence suggests that macrophages,

dendritic cells, neutrophils, NK cells, and mast cells participate in the disease process (Sun et al., 2020).

Macrophages are among the earliest immune cells recruited to the pancreas during insulitis. They secrete pro-inflammatory cytokines such as TNF and IL-1β, which can induce IL-6 production, and they release IL-12, which promotes cytotoxic differentiation of naive T cells (Alleva et al., 2000). Macrophage-derived reactive oxygen species (ROS) can directly contribute to β-cell apoptosis. Through these mechanisms, macrophages help establish an inflammatory milieu within the pancreas that initiates or amplifies β-cell destruction (Carrero et al., 2017). As described earlier, DCs are professional antigen-presenting cells capable of capturing β-cell derived antigens and presenting them to lymphocytes in the pancreatic lymph nodes. By presenting β-cell antigens, DCs can initiate autoimmune T cell responses against the islets (Turley et al., 2003). In addition, DCs secrete cytokines such as IL-12 and IL-15 and upregulate co-stimulatory molecules, all of which enhance the activation of autoreactive T cells and exacerbate T1D pathogenesis (Sun et al., 2020).

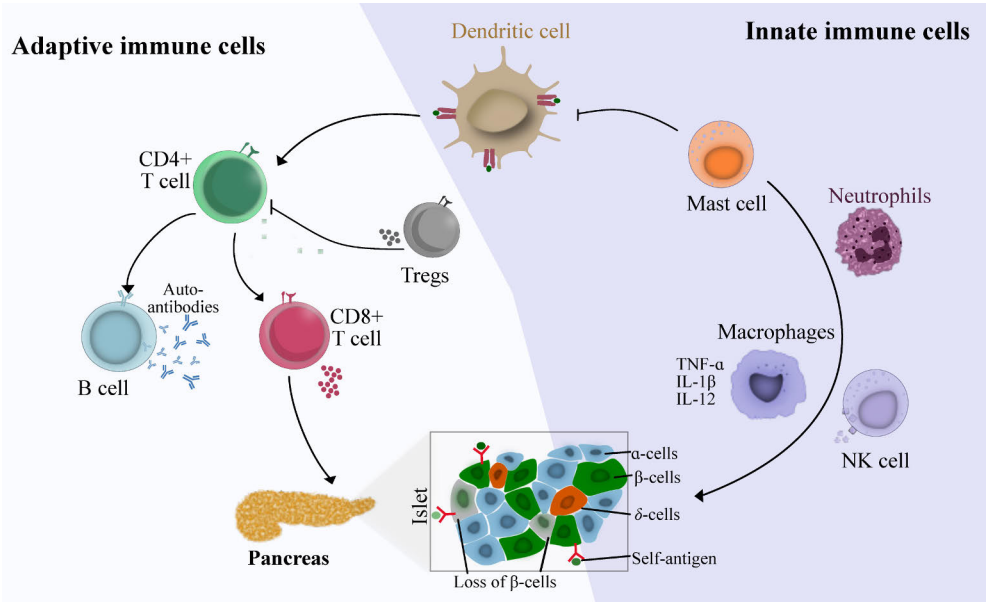


**Figure 7.** Innate and adaptive immune cells contribute to the pathogenesis of T1D. Modified from Sun et al., 2020.

Neutrophils are the most abundant circulating innate immune cells and can be activated by signals from apoptotic β-cells. In NOD mice, neutrophils infiltrate the pancreas during early disease stages (Diana et al., 2013). In humans, reduced

circulating neutrophil numbers and functional abnormalities have been reported in individuals with T1D (Vecchio et al., 2018). Once recruited to the pancreas, neutrophils interact with and activate macrophages and DCs, thereby amplifying local inflammation and contributing to β-cell damage. NK cells interact with antigen-presenting cells and possess the ability to kill target cells directly. In NOD mice, depletion of NK cells delays disease onset, suggesting a role in early β-cell destruction (Poirot et al., 2004). However, because NK cells consist of multiple subsets with distinct functions, their precise contribution to T1D pathogenesis in humans remains unclear. Mast cells produce large amounts of IL-6 and other pro-inflammatory mediators. In mouse models, mast-cell derived IL-6 has been shown to influence the balance between IL-17 producing T cells and tolerogenic Tregs, skewing the immune response toward a more inflammatory phenotype that may promote T1D development (Carlos et al., 2017).

### 2.4.4 Role of adaptive immune cells in T1D progression

Adaptive immune cells including CD4+ T cells, CD8+ T cells, and B cells play a central role in the pathogenesis of T1D (Figure 7). Numerous studies using the NOD mice model have demonstrated the contribution of these immune populations to pancreatic autoimmunity (Sun et al, 2020). In humans, insights are more limited to interpretation of peripheral blood due to the scarcity of pancreatic tissue, which is typically obtained only post-mortem. Nevertheless, these studies consistently show infiltration of B cells, CD4+ T cells, and CD8+T cells within the pancreatic islets of individuals with T1D (Martinov and Fife, 2020).

#### 2.4.4.1 B cells

B cells recognize β-cell autoantigens including insulin and various β-cell enzymes and produce autoantibodies. As discussed earlier, these autoantibodies serve as important biomarkers for predicting autoimmunity and staging disease progression. Beyond antibody production, B cells also function as APCs. In NOD mice, loss of B cell mediated antigen presentation prevents disease. Conversely, enhancing selective antigen uptake by B cells is shown to enhance diabetes in mice. (Hubert et al., 2001, Wong et al., 2004, Bour-jordan and Bluestone 2007). In humans, a loss of anergy in high-affinity insulin-binding B cells have been observed prior to or at the time of diagnosis. Notably, children diagnosed before the age of 7 years exhibit significantly higher numbers of B cells infiltrating the pancreas compared with those diagnosed at older ages (Herold et al., 2024; Leete et al., 2020; Smith et al., 2015).

#### 2.4.4.2 CD8+ T cells

CD8+ T cells are specialized cytotoxic lymphocytes that are consistently enriched in pancreatic insulitis, where they mediate destruction of β-cells through multiple mechanisms (Culina et al., 2018; Damond et al., 2019; Rojas et al., 2018). CD8+ T cells recognize β-cell peptides presented on MHC class I molecules and mediate killing of β cells through perforins, granzymes and Fas-FasL mediated mechanisms (Oshima et al., 2026). Additionally, secretion of IFN-γ also enhances macrophage-mediated inflammatory responses.

Several studies have identified autoantigen-reactive CD8+ T cells in individuals with T1D. In some patients, hyperexpression of HLA class I molecules and the presence of single or multiple autoantigen-specific CD8+ T cells were detected within the same islets, which showed varying degrees of β-cell loss and minimal insulin content (Coppieters et al., 2012). Although similar frequencies of autoreactive CD8+ T cells can be detected in peripheral blood, pancreatic tissue shows a clear enrichment (Culina et al., 2018). Preproinsulin-reactive cytotoxic T cells detected in peripheral blood have been shown to directly kill β-cells (Skowera et al., 2008; Bender et al., 2020). Importantly, autoreactive CD8+ T cells can also be found in healthy individuals, and the factors that trigger their pathogenic activation in the pancreas remain unclear (Bender et al., 2020; Culina et al., 2018;).

#### 2.4.4.3 CD4+ T cells

The strongest genetic association with T1D lies within HLA class II genes, whose primary function is to present antigens to CD4+ T cells (Noble and Valdes, 2011; Walker and Herrath, 2015). Autoreactive CD4+ T cells recognize β-cell antigens and provide help to CD8+ T cells, enhancing their cytotoxicity, and activate B cells to produce autoantibodies. Thus, CD4+ T cells are considered central orchestrators of the adaptive immune response in T1D pathogenesis (Martinov and Fife, 2020).

Multiple studies have demonstrated CD4+ T cell infiltration in pancreatic islets of individuals with T1D. Many of these infiltrating cells are specific for β-cell autoantigens such as proinsulin, GAD, and IA-2 (Babon et al., 2016; Landry et al., 2021; Pathiraja et al., 2014). Autoantigen-specific CD4+ T cells are also detectable in peripheral blood and are elevated in individuals with T1D (Yang et al., 2017). Early studies using the classical Th1/Th2 paradigm identified Th1 cells as the primary pathogenic CD4+ T cell population that promote diabetes in NOD mice (Katz et al., 1995). Human studies similarly reported increased autoreactive Th1 responses producing IFN-γ, alongside reduced IL-10–producing Tregs responses. Balanced IFN-γ and IL-10 responses to islet antigens were associated with healthy immunity in autoantibody-negative first-degree relatives of T1D patients (Arif et al., 2004; Petrich de Marquesini et al., 2010). With the identification of additional Th

subsets, Th17 cells and Tregs have also been implicated in T1D. IL-17 was shown to induce IL-1β and IFN-γ, promoting β-cell apoptosis (Arif et al., 2011). Increased IL-17$^+$ T cells and IL-17 secretion have been reported in children with new-onset T1D (Honkanen et al., 2010; Marwaha et al., 2010). Upregulated Th17 responses and impaired Tregs function were observed specifically in pancreatic-draining lymph nodes but not in peripheral blood of patients with long-lasting T1D (Ferraro et al., 2011).

Tfh cells that produce IL-21 and support B cells for antibody production, are also elevated in the peripheral blood of individuals with T1D and may contribute to disease progression (Ferreira et al., 2015; Kenefeck et al., 2015; Viisanen et al., 2017). Blocking CD80/CD86 co-stimulation reduced circulating Tfh cells in T1D patients, and baseline Tfh phenotypes correlated with clinical response (Edner et al., 2020). Tregs exhibit substantial functional heterogeneity and plasticity toward other Th lineages (Dean et al., 2020; Vecchione et al., 2021). Although overall Tregs frequencies in peripheral blood may be comparable between T1D patients and controls, T1D individuals show increased frequencies of IFN-γ$^+$ FOXP3$^+$ Tregs, suggesting altered function (McClymont et al., 2011).

### 2.4.5 Early immune cell changes during T1D progression

As discussed above, multiple studies have shown that immune dysregulation plays a central role in T1D. Before the development of the current staging model, most knowledge came from peripheral blood samples collected at clinical diagnosis and followed longitudinally post onset, or from cadaveric pancreatic tissue representing late disease. These approaches offered limited insight into the early immune events that initiate T1D.

Longitudinal cohort studies prior to seroconversion have been crucial for filling this gap. By recruiting genetically at-risk individuals or those with early Aab, these studies have revealed immune alterations that precede seroconversion and contribute to disease progression (Ferreira et al., 2014, Kallionpää et al., 2014). Large consortia have used these insights to design prevention trials. TrialNet, for example, demonstrated that anti-CD3 therapy (teplizumab) can delay progression from stage 2 to clinical T1D by 2–3 years, although responses vary between individuals (Herold et al., 2019). This highlights the need for accurate risk stratification and a deeper understanding of early immune changes that influence treatment responsiveness.

Birth cohort studies following genetically predisposed children from infancy to diagnosis have shown that immune cell changes can be detected even before the first Aab appears. Bulk transcriptomics first revealed these early alterations (Bediaga et al., 2022; Ferreira et al., 2014; Kallionpää et al., 2014; Kallionpää et al., 2019; Lin et al., 2023), and more recent single-cell technologies have identified subset-specific

immune signatures at diagnosis of T1D (Honardoost et al., 2024; Niederlova et al., 2025; Okamura et al., 2025; Zhong et al., 2024). Recent studies also showed that early immune trajectories of children differ depending on the first-appearing autoantibody (Lin et al., 2023; Starskaia et al., 2024).

Study I of this thesis identified subset-specific gene expression changes and gene regulatory networks in CD4+ T cells prior to seroconversion using longitudinal birth cohort. Viral response and interferon signaling pathways were affected in multiple CD4+ T cell subsets in children progressing to disease (Biradar et al., 2025).

Understanding these early immune alterations is essential for developing targeted interventions to delay or prevent T1D.

# 3 Aims

The overall goal of this PhD study was to study gene regulation of human CD4+ T cells by characterizing transcriptomic changes in CD4+ T cells during T1D progression and investigating their early differentiation into Th17 cells.

The presented study in this thesis has the following three specific aims:

(1) To understand changes in blood CD4+ T cells during an early stage of T1D before appearance of autoantibodies (seroconversion).

(2) To study the role of FOS like transcription factors FOSL1 and FOSL2 in CD4+ T cell polarization towards Th17 differentiation.

(3) To identify enhancers specifically active during initial stages of CD4+ T cells polarization towards Th17 differentiation.

# 4 Materials and Methods

## 4.1 Ethics statement (Study I, II and III)

The study I protocol was approved by the ethics committee of the participating centers in Trial to Reduce Insulin Dependent Diabetes Mellitus (IDDM) in Genetically at Risk (TRIGR) cohort study.

Collection of human umbilical cord blood from healthy neonates (study II and III) was approved by the Ethics Committee of the Hospital district of Southwest Finland (Pöytäkirjanote NRO 10/1998) in line with the 1975 Declaration of Helsinki.

## 4.2 Materials and methods for study I

### 4.2.1 T1D cohort samples

Samples were obtained from the TRIGR study cohort. The TRIGR study population includes newborn infants with at least one family member affected by T1D and had one of the following HLA genotypes: (a) HLA-DQB1*0302/DQA1*05-DQB1*02; (b) HLA-DQB1*0302/x (x not DQB1*02, DQB1*0301, or DQB1*0602); (c) HLA-DQA1*05-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603); (d) HLA-DQA1*03-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603). The detailed study design of the TRIGR study cohort have been described earlier (TRIGR Study Group. 2007). The cases include 11 at-risk children who later progressed to clinical disease. The control samples were collected from HLA-, age- and sex-matched children who neither progress to beta cell autoimmunity nor the clinical disease. All the children with T1D had developed the disease at an early age (1.6–4.3 years, mean 2.5 years) and they had seroconverted by the age of 2 years, thus composing a cohort of fast progressors. The study protocol was approved by the ethics committee of the participating centers. The PBMCs were isolated from peripheral blood using density gradient centrifugation, stored at -150$^{o}$C and shipped for further analysis to respective laboratories.

### 4.2.2 Single-cell RNA-sequencing with cell multiplexing

CD4+ T cells were isolated from PBMCs using DynabeadsTM CD4+-positive isolation kit (Invitrogen #11331D). CD4+ T cells of children who rapidly progressed to T1D and their controls was processed for for scRNA sequencing using Chromium Single Cell Kits (10X Genomics Single cell 3' Reagent Kit V3.1). Cell multiplexing using human cell hashing antibodies from BioLegend (TotalSeq$^{TM}$-B0251-B0260, anti-human Hashtag1-10) was used to stain up to 10 case and control CD4+ T cell samples. A total of half a million CD4+ T cells from each sample were independently stained with human cell hashtag antibody as per manufacturer's protocol.

Stained cells from each pair (case and control) were pooled together in equal proportions as one multiplexed sample and loaded onto Chromium Next GEM Chip G. For each run 33,000 cells at a cell concentration recommended by 10X Genomics was loaded to achieve 20,000 single cells for the analysis. Cells in gel in emersion (GEM) were lysed for reverse transcription and complimentary DNA (cDNA) amplification. Full-length cDNA along with cell barcode identifiers were PCR-amplified. Two different cDNA sequencing libraries were prepared from reverse-transcribed polyadenylated transcripts of each cell and from cell hashtag oligo bound to the same cell, respectively, utilizing dual indexing. Sequencing of cDNA libraries was performed with Illumina NovaSeq 6000 at sequencing depth of 20,000 and 5,000 reads per cell for gene expression and cell hashtag libraries, respectively. The data were processed using Cell Ranger pipeline version 3.0.1. Computational demultiplexing of pooled samples was performed and single-cell transcriptome data were obtained.

### 4.2.3 Analysis of scRNA-seq

In study I, the bioinformatic analysis were conducted in collaboration with Prof. Laura Elo's research group. Only cells identified as singlets in the demultiplexing of hashtag oligos were retained in the analysis. Additionally, low-quality cells expressing less than 200 or more than 3,000 genes or having more than 10% mitochondrial genes were filtered out. All cells were analyzed together. Normalization, identification of highly variable features, scaling, dimensionality reduction with PCA, integration with reciprocal PCA, and clustering were performed by the Louvain method using Seurat (v.4.0.1) in R (v.4.1.0). Clusters were annotated automatically with Azimuth (v.0.3.2) using a human PBMC reference (Hao et al., 2021). Cells annotated as monocytes, B cells or dendritic cells were removed from the analysis. After removal of the contaminating cell types, clustering was repeated using resolution of 1.2, and clusters were annotated manually using conserved marker genes. Pseudo-bulks for differential expression analysis were created by

aggregating the counts across all cells in one sample in one cell type for each gene using muscat (v.1.6.0).

### 4.2.4 Longitudinal analysis

A linear mixed effects model was fitted to the data separately for each gene, with the gene expression level as the dependent variable. Disease status, age, their interaction, and sex were treated as fixed effects and the case-control pair as random effect. The mixed effects modelling was implemented using lmerTest (v.3.1-3) R package. Correction for multiple testing was done using Benjamini-Hochberg adjustment. The analysis was performed separately for each cell type.

### 4.2.5 Regulon analysis

Gene regulatory networks governed by regulons (TFs and their direct target genes), were predicted and analyzed using the 'single cell regulatory network inference and clustering' (SCENIC; v.0.12) method (Van de Sande et al., 2020). First, candidate regulatory modules were inferred from co-expression patterns between genes. These indirect targets were removed using transcription factor motif information in a pre-calculated database provided with the SCENIC tool. The database used for this pruning step was the 500 bp upstream and 100 bp downstream of the transcription start site. The activity of the discovered regulons was measured in each cell and used for clustering in a network. The regulon activity was further binarized and contrasted with the scRNA-seq clustering results. The activity of each regulon was plotted in the pre-calculated UMAP projection for interpretation. A gene regulatory network map was generated using Cytoscape (v.3.9.1) for those regulons showing statistically significant differences ($p<0.01$ Wilcoxon signed-rank test) in their activity between the cases and controls using. Each transcription factor was assigned with at most 10 most important targets based on the SCENIC analysis for visualization.

### 4.2.6 Time point-wise differential expression analysis

To test for differences in expression between cases and controls at 3, 6, 9, 12 and 18 months time points, reproducibility-optimized test statistic (ROTS; v.1.32.0) R package was used (Suomi et al., 2017). The pseudo-bulk data where the gene expression from each different cell type cluster were combined to get the cell type-specific bulk expression of each gene in each sample for ROTS analysis. The analysis was performed separately for each cell type without any additional variables.

### 4.2.7 Pathway enrichment analysis

Gene set enrichment analysis (GSEA) for the ranked gene list of LME and ROTS analysis for each cell type, ClusterProfiler R package was used. The gseGO function was applied on the DE genes ranked by either LME coefficient or log2(foldchange) for LME or ROTS analysis using following setting: OrgDb = org.Hs.eg.db, ont = "BP," keyType = "SYMBOL," pAdjustMethod = "BH," pvalueCutoff = 0.05, exponent= 1, eps = 1e-300, nPerm = 10, 000, minGSSize = 10, and maxGSSize = 500. The *simplify* function was used to simplify the enriched terms. The top 15 significantly enriched terms (ranked by NES) and the cell type-specific terms of each cell type were selected for visualization.

## 4.3 Materials and methods for study II and III

### 4.3.1 Human CD4+ T cell isolation and Th17 differentiation (Study II and III)

The umbilical cord blood of healthy neonates was used to isolate human cord blood mononuclear cells (CBMCs) (Turku University Central Hospital, Turku, Finland) using the Ficoll-Paque density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare). Naive CD4+ T cells were purified using CD4+ Dynal positive selection beads (Dynal CD4 Positive Isolation Kit; Invitrogen). CD4+ T- cells were stimulated with plate-bound α-CD3 (Beckman Coulter, Cat. no. IM1304) and soluble α-CD28 (Beckman Coulter, Cat. no. IM1376) in X-vivo 20 serum-free medium (Lonza). X-vivo 20 medium was supplemented with L-glutamine (2 mM, Sigma-Aldrich) and antibiotics (50 U/ml penicillin plus 50 μg/ml streptomycin; Sigma-Aldrich). Th17 polarization was induced using a cytokine cocktail of IL-6 (20 ng/ml; Roche), IL-1β (10 ng/ml) and TGF-β (10 ng/ml), in the presence of neutralizing anti- IFN-γ (1 μg/ml) and anti-IL-4 (1 μg/ml) to block Th1 and Th2 differentiation, respectively. For the differentiation control, activated T cells (Th0) were used, which were obtained by stimulating naive CD4+ T cells with α-CD3 and α-CD28 in the presence of neutralizing antibodies, but without the polarizing cytokines. All cytokines and neutralizing antibodies used in the study were purchased from R&D Systems unless otherwise stated. All cultures were maintained at 37°C in a humidified atmosphere of 5% (v/v) $CO_2$ /air. Th17 cells were harvested either at 24, 48 or 72 h of differentiation.

### 4.3.2 RNAi silencing (Study II)

CD4+ T cells from umbilical cord blood were suspended in Opti-MEM I (Invitrogen) and transfected with the respective gene-targeting siRNA (STAT3, BATF, FOSL1

and FOSL2) using the Lonza nucleofection technique with the U-14 program on the Amaxa NucleofectorTM device. Control cells were treated with the same amount of non-targeting or Scramble siRNA (SCR) (Sigma). siRNA transfected cells were rested in RPMI media supplemented with pen/strep, L-glutamine and 10% FCS. Cells were subsequently activated and cultured under Th17 conditions.

### 4.3.3 CRISPR-Cas9-mediated knock-out (Study III)

Guide RNAs (gRNAs) were assembled *in vitro* with Cas9 protein. CRISPETa design tool was used for designing crRNAs and synthesized by IDT, together with tracrRNA (Alt-R CRISPR-Cas9 tracrRNA, IDT), were reconstituted to 160 μM and mixed at equal molar ratio. The mixture was incubated at 37 °C for 30 min to generate 80 μM gRNA. gRNA was then combined with an equal volume of 40 μM recombinant *S. pyogenes* Cas9-NLS protein (QB3 MacroLab, UC Berkeley) and 1 μL of 100 μM ssODN enhancer (IDT), followed by incubation at 37 °C for 10 min to form 20 μM CRISPR-Cas9 ribonucleoprotein (RNP).

For genomic deletion, paired RNP complexes were prepared using crRNA-L4 (5′-ACTGCTCTCTGCTAGCCTGG-3′) and crRNA-R4 (5′-TGGTCTATAGCCAGGACACT-3′). Control RNPs were generated using either a non-targeting crRNA (NC1, IDT: 5′-CGTTAATCGCGTATAATACG-3′) or an AAVS1-targeting crRNA (IDT: 5′-CCTCTAAGGTTTGCTTACGA-3′).

Four million $CD4^+$ cells were nucleofected with the RNP complexes, rested for 24 h in RPMI supplemented with 10% serum, and subsequently cultured under Th17 conditions. At 72 h post-activation, 80 μL of cells was collected for genomic DNA extraction (QuickExtract DNA Extraction Solution, Epicentre). An 885-bp region spanning the CRISPR target site was amplified using KAPA HiFi HotStart PCR (Roche) with primers: Forward 5′-AGCCAGGGCTGTGTTATTC-3′ and Reverse 5′-ACTAACTGCAGCCCAACATAG-3′. PCR products were resolved on a 1.2% agarose gel, purified (GeneJET Gel Extraction Kit, ThermoFisher), quantified (NanoDrop 2000), and subjected to Sanger sequencing (FIMM Sequencing Laboratory, University of Helsinki) using primer 5′-GACACGCTGTTCCACTTAATT-3′.

### 4.3.4 Luminex assay (Study II and III)

IL-17A levels secreted in the supernatant were measured by Luminex assay (human IL-17A DuoSet ELISA kit, R&D Biosystems DY317- 05, DY008 and Luminex 200 by Luminex xMAP technology) according to the manufacturer's instructions. The concentrations were normalized using cell count data obtained by flow cytometry.

### 4.3.5 Flow cytometry (Study II and III)

Th17 cells were harvested for 72 h, washed with flow cytometry staining buffer (1%FBS in PBS) and further incubated with PE-labeled anti-CCR6 antibody with isotype controls for 15 min, at 4ºC in the dark. Stained cells were washed for two times and resuspended in before processing in to flow cytometry. Samples were analyzed using a LSRII or Fortessa flow cytometer (BD Biosciences). Live cells were gated based on forward and side scattering. The acquired data were analyzed with FlowJo (FlowJo, LLC).

### 4.3.6 Luciferase assay (Study III)

$CD4^+$ cells were polarized to Th2 (control) or Th17 conditions for 72 h and transiently nucleofected with the pGL4.10 promoter-luciferase reporter plasmid (Promega). After a 48 h resting period, cells were reactivated under Th2 or Th17 conditions for 24 h, lysed in passive lysis buffer (Dual-Luciferase Reporter Assay, Promega), and luciferase activity was quantified according to the manufacturer's protocol. Firefly luciferase signals were normalized to co-transfected pGL4.74 Renilla luciferase and expressed as fold-change relative to the empty pGL4-minP construct. Reporter plasmids containing the RORA enhancer region were synthesized by GeneScript.

### 4.3.7 Western blotting (Study II and III)

T cells were lysed using RIPA buffer (Pierce, Cat. no. 89901), supplemented with protease and phosphatase inhibitors (Roche) and sonicated using Bioruptor UCD-200 (Diagenode, Seraing, Belgium). Sonicated cell lysate was centrifuged at 14000 RPM for 20 min at 4 ◦C and supernatant was collected. Protein concentration of the sample was estimated by DC protein assay (BioRad) and heated with 6× Laemmli buffer at 96 ◦C for 5 min. Samples were loaded on gradient Mini-Protein TGX precast loading gel (BioRad) and transferred to PVDF membrane (Trans-Blot Turbo transfer packs, Bio-Rad). The following antibodies were used: anti-RORα (Santa Cruz, sc- 518,081), anti-β-Actin (Sigma, A5441) and HRP-conjugated anti- mouse IgG (Santa Cruz, sc-516,102) as secondary antibody. For FOSL1 and FOSL2, the following Ab were used: anti-FOSL1 (Cell Signaling Tech., cat no. 5281), anti-FOSL2 (Cell Signaling Tech., cat no. 19967), and anti-β-actin (Sigma-Aldrich, cat no. A5441). HRP- conjugated anti-mouse IgG (Santa Cruz Biotechnology, cat no. sc-2005) and anti-rabbit IgG (BD PharMingen, cat no. 554021) were used as secondary Ab.

### 4.3.8 Cellular fractionation (Study II)

Cell pellets of Th0 and Th17 cultures (24 and 72 h) were lysed and fractionated into cytoplasmic and nuclear components using a NE-PER nuclear and cytoplasmic extraction reagent kit (Thermo Fisher Scientific, cat no. 78833) by following the manufacturer's instructions. Extracts were then analyzed by western blotting. The localization of FOSL proteins was determined using anti-FOSL1 (Cell Signaling Tech., cat no. 5281) and anti-FOSL2 (Cell Signaling Tech., cat no. 19967) antibody. Anti-GAPDH (HyTest, cat no. 5G4) and anti-LSD1 (Diagenode, cat no. C15410067) antibodies were used to mark the cytoplasmic and nuclear fractions, respectively.

### 4.3.9 Immunoprecipitation (Study II)

Immunoprecipitation (IP) with specific antibody was performed using a Pierce MS-compatible magnetic IP kit (Thermo Fisher, cat no. 90409). Cell pellets from 72 h cultured Th17 cells were lysed in appropriate volumes of lysis buffer (provided in the kit), and also supplemented with protease and phosphatase inhibitors (Roche). Lysates were estimated for protein concentration (DC protein assay; Bio- Rad). IP was performed using the following Ab: anti-FOSL1 (Santa Cruz Biotechnology, cat no. sc-28310), anti-FOSL2 (Cell Signaling Technology, cat no. 19967), mouse IgG (negative control for FOSL1: Cell Signaling, cat no. 5415), and rabbit IgG (negative control for FOSL2; Cell Signaling Technology, cat no. 2729). Equal amounts of Ab (µg) were used for each control IgG and FOSL IP reaction. All Ab were pre-incubated with 60 µL of protein A/G beads for 4−5 h to form antibody−bead complexes. Protein lysates (1 mg/IP reaction) were first pre-cleared with control IgG−bead complexes for 3 h. The pre-cleared lysates were then incubated overnight with FOSL1/FOSL2 antibody−bead complexes (test IP) or the corresponding control IgG−bead complexes (negative IP control). The pull-down fractions were washed (following the manufacturer's protocol) and eluted with an elution buffer. Test and control IP samples were eluted in equal volumes of buffer. The eluted protein was vacuum-dried for MS analysis or run for western blotting. The Ab used for IP−WB are as follows: anti-FOSL1 (Santa Cruz Biotechnology, cat no. sc-28310), anti-FOSL2 (Cell Signaling Technology, cat no. 19967), anti-RUNX1 A-2 (Santa Cruz Biotechnology, cat no. sc-365644); anti-JUNB C-11 (Santa Cruz Biotechnology, cat no. sc-8051); anti-SIRT1 (Cell Signaling Technology, cat no. 2496); and anti-JUN (BD Biosciences, cat no. 610326). Conformation-specific rabbit HRP (Cell Signaling Technology, cat no. 5127) and mouse HRP (Cell Signaling Technology, cat no. 58802) were used as secondary Ab.

### 4.3.10 ChIP-seq analysis for BATF and FOSL (Study II)

Th17 cells are cultured for 72 h. Chromatin was prepared from 40–50 million cells using Diagenode Chromatin shearing optimization kit (Cat. no. C01010055) and further subjected to sonication using Bioruptor sonicator (Diagenode) to obtain chromatin fragments. FOSL1 (Santacruz Biotechnology, Cat no. sc-28310), FOSL2 (Cell Signaling Tech, Cat. no. 19967) or BATF (Cell Signaling, Cat. no. 8638) antibody was pre-incubated with magnetic beads for crosslinking (Dy- nal Biotech/Invitrogen, Cat. no. 112.04). DNA libraries were prepared using two biological replicates of each TF ChIP and sequenced on HiSeq4000 or Miseq (Fasteris Life Sciences, Switzerland). The quality control was performed with FastQC (v. 0.11.4). The reads were mapped to the hg38 reference genome using Bowtie2 (v. 2.3.3.1). Reads with mapping quality <30 was filtered out. Peaks were called using MACS2 (v. 2.1.0), and reproducible peaks were identified using IDR (36) with an FDR cut-off of 0.01. R packages ChIPpeakAnno (v. 3.21.7) and EnsDb.Hsapiens.v86 (v. 2.99.0) were used to annotate the peaks and identify peaks common to all three transcription factors with a minimum overlap of 200 bp. The number of peaks common to the transcription factors are reported. Enriched transcription factor binding site motifs within the peaks were identified by Homer (v. 4.11) using both *de novo* and known motifs. A 200-bp region size was used for motif finding.

### 4.3.11 TaqMan quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) (Study II and III)

For qRT-PCR, RNA was isolated (RNeasy Mini Kit, #74106, QIAGEN) and treated in-column with DNase (RNase-Free Dnase Set, #79254, QIAGEN) for 15 min. The removal of genomic DNA was ascertained by treating the samples with DNase I (Invitrogen, #18068–015) before cDNA synthesis with SuperScript II Reverse Transcriptase (Invitrogen, #18064014). RT-qPCR was performed using KAPA Probe Fast Rox Low master mix (KAPA Biosystems, #kk4718) and amplification was monitored with QuantStudio 12 K Flex Real-Time PCR System (ThermoFisher Scientific). The Ct values were normalized against the signal acquired with EF1A. The following primers were used:

IL17A (Forward: 5′- TGGGAAGACCTCATTGGTGT-3′and Reverse: 5′-GGATTTCGTGG- GATTGTGAT-3′), RORA (ThermoFisher Scientific, Cat# 4331182, Assay ID: Hs00536545_m1), EF1A (Forward: 5′-AGCAAAAACGACCCACCA- 3′and Reverse: 5′-GCCTGGATGGTTCAGGATAA-3′).

### 4.3.12 SNP enrichment analysis (Study II and III)

SNP enrichment analysis was performed with snpEnrichR (v.0.0.1) R package. For SNP enrichment analysis, disease-associated SNPs, we used disease-associated SNPs from NHGRI-EBI GWAS catalogue or ImmunoChip database. SNPs from studies with meta-analysis of more than one disease and from populations other than Caucasian were excluded from further analysis, and correlated SNPs were clumped (distance = 1000 kb, LD $r^2 = 0.8$). Random SNP sets matching the disease-associated SNPs were produced using SNPsnap server with default parameters except distance = 1000 kb, LD buddies ±20%, r2 = 0.8. Proxy SNPs for both disease-associated and random SNPs were calculated using Plink (v1.90b6.27) from 1000 genomes EUR population.

The enrichment null distributions are assessed by counting the overlaps of SNPs with random locations of size equal to enhancer sets from the genome with 1000-fold repetition. The overlap method excludes the telomeres and centromeres as they are not known to hold any regulatory sites. SNPs and their proxies (distance within 100 kb and r2 > 0.8, determined from 1000 genomes European population) overlapping the peaks, were identified and annotated to the nearest neighbor gene using ChIPpeakAnno. SNPs overlapping known transcription factor motifs were identified using annotatePeaks.pl from Homer (v.4.10). Motifs were searched within a 30-bp region around each SNP coordinate. For the heatmap visualizing the cis-eQTL effects of the SNPs overlapping transcription factor binding site (TFBS), euclidean distances of both rows and column were clustered using Ward's minimum variance method.

### 4.3.13 DNA affinity precipitation assay (DAPA) (Study II)

DNA affinity precipitation assays were performed as previously described (Tripathi et al., 2017) with minor modifications. Biotinylated sense and non-biotinylated antisense oligonucleotides (IDT) were annealed to generate probes containing FOSL1, FOSL2, or BATF binding motifs, with or without the SNP variant. BATF-specific and mutant probes served as positive controls. Neutravidin beads (Pierce) were washed four times with buffer A (10 mM HEPES pH 7.9, 60 mM KCl, 2 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 1 mM DTT, protease/phosphatase inhibitors). Annealed probes were incubated with 25 µl beads in 200 µl buffer A for 1.5 h at 4 °C with rotation, followed by four washes.

Nuclear extracts from Th17 cells cultured for 72 h (Pierce Nuclear and Cytoplasmic Extraction Kit) were diluted in buffer 2 (10 mM HEPES pH 7.9, 2 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 1 mM DTT, protease/phosphatase inhibitors) to reduce KCl concentration. Extracts were pre-cleared with unconjugated beads for 1.5 h at 4 °C, then incubated with probe-conjugated beads

for 4 h at 4 °C. After four washes with buffer A, bound proteins were eluted by heating beads at 95 °C for 5 min in 2× SDS buffer (125 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 100 mM DTT). FOSL1, FOSL2, and BATF were detected by western blot using rabbit monoclonal antibodies (Cell Signaling Technology).

### 4.3.14 ATAC-seq (Study III)

Differentiating Th17 cells and matched control Th0 from two donors were collected at 0, 2, 4, 24, 48, and 72 h. ATAC-seq libraries were generated following the protocol described earlier (Khan et al., 2022). Amplified libraries underwent a two-step SPRI bead purification, using a 0.4× bead-to-DNA ratio to remove large fragments (flow-through retained), followed by a 1.4× ratio in which bead-bound DNA was collected. Libraries were eluted in 20 µl Buffer EB (QIAGEN) and assessed for size distribution and quality using an Agilent Bioanalyzer. Sequencing was performed on an Illumina HiSeq 2500, with all samples distributed across all lanes to minimize lane-specific batch effects.

Raw sequencing reads were evaluated with FastQC (v0.11.4). Adapter trimming was performed using Trim-Galore (v0.4.5), and trimmed reads were aligned to the human hg38 reference genome using Bowtie2 (v2.3.3.1). Duplicate reads were marked with Picard MarkDuplicates (v2.17.8). Peak calling was carried out with MACS2 (v2.1.0), and reproducible peaks between biological replicates were identified using IDR (FDR $\leq 0.05$). A union set of all reproducible peaks across samples was defined as the reference feature set for downstream analyses.

Read quantification across genomic regions was performed using featureCounts (Rsubread), and raw counts were normalized using the TMM method. Differential accessibility analysis was conducted with the Limma package, defining regions with fold-change $> 2$ and FDR $\leq 0.01$ as significantly altered. Genomic annotation of accessible regions was performed using HOMER (v4.9).

### 4.3.15 Histone ChIP-seq (Study III)

Cells were activated under Th0 and Th17 condition for 0.5 and 72 h. ChIP assay was performed using H3K4me1 (Diagenode, C15410194), H3K4me3 (Diagenode, C15410003) and H3K27ac (Diagenode, C15410196) antibodies as described. The quality of the raw data was checked with FastQC (v.0.11.3) and MultiQC (v.1.5). The library complexity for all the samples fulfilled the quality criteria recommended by ENCODE. The reads were aligned to hg38 reference genome using Bowtie. Most samples had around 20 million uniquely mapped reads. Cross correlation analysis found an enrichment in all the antibody samples but not in any input samples, suggesting that ChIP experiments were successful. Library complexity was

determined with preseq (v.2.0.3). Only reads of mapping quality >30 was used in the downstream analysis. Duplicates were removed with Picard tools (Picard), and samples were then down- sampled to the median depth.

### 4.3.16 Identification of enhancers and super-enhancers (Study III)

Enhancers were predicted with ChromHMM (v.1.20) with five chromatin states. Two result states were manually defined to correspond to weak and strong enhancers and were included in the subsequent analysis. For predicting SEs, ROSE (v.0.1) was used with H3K27ac as ranking signal. To identify enhancers unique to a cell type (Th0 or Th17) or stage of differentiation (0.5 h or 72 h), we used MAnorm (v.1.1.4). We preferred MAnorm over bedtools-based subtractions because the former also provides statistical measures of confidence. Log2FC > 0.6 and $p < 0.01$ cut off were used for significance. Enhancers from different replicates were merged such that the enhancers that were found in at least two replicates were retained, and their boundaries were the union of all the replicates where the enhancer was found.

### 4.3.17 Transcription factor binding site analysis (TFBS) (Study III)

TFBSs overrepresentation on the enhancers was examined using the FMatch tool at the TRANSFAC database. As a background for calculating overrepresentation, a randomly generated gene set of approximately the same size was taken. A custom profile was generated using the matrices corresponding to the TFs expressed in T cells during early differentiation: 658 motifs. The enrichment was calculated using binomial test. The p-value was corrected in R version 4.2.1 using Benjamin and Hochberg method at FDR < 0.05 considered significant.

# 5 Results

## 5.1 Single cell RNA-seq analysis of longitudinal CD4+ T cell samples reveals cell-type-specific changes during early stages of T1D

### 5.1.1 scRNA-sequencing identified CD4+ T cell subsets

To investigate changes in CD4+ T cells during development of T1D from birth until the appearance of islet autoantibodies (seroconversion), we utilized scRNA sequencing. PBMCs were collected from 11 pairs of case and control children with 73 longitudinal samples collected at regular interval such as 3, 6, 9, 12, 18 and 24 months until seroconversion. All case children seroconverted by the age of 2 years and developed T1D by age of 5 years (Study I/ Table 1; Figure 1a). CD4+ T cells were isolated from PBMCs. Individual sample tagged with cell hashtag antibodies allowed sample multiplexing. The multiplexed sample was processed for 10X Genomics sc-sequencing while demultiplexing analysis was performed to obtain data from each pooled sample (Study I/ Figure 1b). Quality control filtering ensured retention of high-quality single cell for data analysis. Unsupervised clustering of cells from all the samples combined detected 23 cluster in 99,263 single cells. Clusters with similar expression of top maker genes in the UMAP vicinity were merged to obtain 13 final clusters. Three clusters had fewer cells with cells over-represented from few samples. Remaining 10 cluster were annotated (Study I/ Figure 2a-b). Three clusters of naive phenotypes, two clusters of central memory, two cluster of regulatory and three clusters of effector helper cells were annotated using gene markers (Study I/ Figure 2c).

### 5.1.2 Cell type proportions were similar in cases and controls

Differential abundance analysis was performed using linear mixed effect (LME) modelling to test cell type proportion differences withing ten cell types of CD4+ T cells. Each cell subtype was similarly represented between cases and controls and no significant differences were observed (Study I/ Figure 2d-e).

### 5.1.3 Differential gene expression analysis identified genes associated with development of disease

Differential gene expression using LME modelling was performed to identify genes associated with case-control status or age withing each CD4+ T cell subtype. The interaction of status and age as a variable was additionally included. The analysis identified 285, 785 and 172 genes associated with the status, age and status-age interaction term respectively ($p < 0.001$). Genes associated with case-control status were mainly identified in effector cell type whereas age-associated genes were identified predominantly in the naive and central memory cells (Study I/ Figure 3a). After multiple correction testing, eight genes were found to be differentially expressed (DE) between cases and controls: *PSME4, NTPCR, MFHAS1, IQGAP2, ZMYM3, HSD17B10, ABCB7* and *SLC35E4.* These genes were DE mainly within CD69+ naive cells and central memory cells (Study I/ Figure 3b-c). Except for *IQGAP2*, expression difference for other DE genes varied between the cell types even though without statistical significance. PSME4 was the only gene downregulated in cases while others were upregulated in cases. *PSME4, NTPCR, SLC35E4, MFHAS1* and *IQGAP2* have some known associations while other three genes did not have direct association with diabetes. These DE gene changes between cases and controls was validated by applying an orthogonal method using ROTS analysis.

### 5.1.4 Pathways affected in children progressing to T1D

GSEA was performed on the ranked list of case-control affected genes from LME analysis. Notably 'response to virus' pathways was enriched in naive T cells, central memory T cells, Th17 cells and naive Tregs. Additionally, 'interferon mediated signaling pathway' was enriched in naive and central memory cells. Cell type specific terms such as 'interferon beta production' and 'interleukin 10 production was enriched in naive Tregs and effector Tregs respectively (Study I/ Figure 3d). Many of the pathways including antiviral and interferon-related pathways were also identified in GSEA of genes ranked based on ROTS analysis.

### 5.1.5 Gene regulatory networks in children progressing to T1D

SCENIC analysis was performed to study cell type specific gene regulatory networks (referred as regulon: transcription factor along with their target genes) in our data. A total of 245 regulons were identified across 10 cell types. The regulon specificity score was binarized to indicate whether regulon is active or inactive in each cell type. The top 10 most active regulons per cluster resulted in 61 unique regulons. A cell

type specific binary activity was calculated for regulons and motif binding site of those was analyzed (Study I/ Figure 4a-b). Regulons TBX21, MAF and FOSL1 were active in Th1, Th2 and Th17 cells, respectively.

Differential activity for 12 regulons was observed between cases and controls in specific cell types. Pathway enrichment analysis revealed the biological process associated with these regulons (Study I/ Figure 5a). Four regulons (MAFA, ZNF543, PRDM1, ZSCAN18) were present in Th17 cells, four (MAFG, ZNF143, NFIC, DLX2) in MALAT1$^{+}$ naive T cells while other four (GATA3, PAX8, SREBF1, and REST) regulons were differentially active in naive T cells, CD69+ naive T cells, Th1 cells and effector Tregs, respectively. The target genes of these regulons were unique as well as shared among regulons. The highest number of shared target genes were noted for PRDM1 and GATA3 regulon with increased activity in Th17 cells and decreased activity in naive T cells in cases compared to controls, respectively (Study I/ Figure 5b-e).

## 5.2 FOSL1, FOSL2 and BATF regulate early human Th17 differentiation

### 5.2.1 FOSL1 and FOSL2 regulate early human Th17 differentiation

To study FOSL1 and FOSL2 gene expression, we analyzed RNA-seq gene expression profile (Tuomela et al., 2016) during early human Th17 cell differentiation. Both FOSL1 and FOSL2 showed differential gene expression, and their levels were significantly upregulated in Th17 polarized cells as compared to T cells activated in the absence of Th17 polarizing cytokines (Study II/ Figure 1A). Immunoblotting of FOSL protein from *in vitro* differentiation of Th17 cells showed highest expression at 24h timepoint (Study II/ Figure 1B). Compared to Th0 cells as control, Th17-polarizing cytokines specially IL-6 and IL-1β enhanced the expression of both FOSL proteins (Study II/ Figure 1C). STAT3 knockdown experiments further demonstrated that FOSL2, but not FOSL1, is a direct downstream target of STAT3, correlating with STAT3 occupancy at the FOSL2 promoter (Study II/ Figure 1C-D). To determine potential involvement of FOSL proteins in Th17 differentiation we silenced these proteins and evaluated their effect on Th17 differentiation. Interestingly, individual FOSL1 or FOSL2 knockdown (KD) significantly increased IL-17 secretion while double knockdown (DKD) enhanced the effect (Study II/ Figure 2A-E). Conversely, overexpression (OE) of FOSL1 and FOSL2 (individually and in combination) resulted in suppression of IL-17 production, further supporting their negative regulatory role (Study II/ Figure 2F-H).

### 5.2.2 FOSL protein modulates Th17 gene expression program

The individual FOSL1, FOSL2 KD and combined DKD was performed in CD4+ T cells, whereafter the cells were differentiated towards Th17 lineages. Th17 cells were harvested at 24 h and 72 h of Th17 polarization to access gene targets of FOSL at early and late timepoints. RNA-sequencing followed by differential gene expression analysis revealed 466, 1529 and 2000 DE genes at 24 h, while 315, 150 and 1500 genes were DE at 72 h compared to non-targeting control (FDR < 0.1). The double knockdown of both genes altered a higher number of genes at both timepoints. An OE of individual FOSL1, FOSL2 and combined double over expression (DOE) at 72 h of Th17 polarization resulted in identification of 30, 352 and 521 DE genes (FDR < 0.1). The genes co-regulated by FOSL are identified in the heatmap by comparing individual vs double KD and OE (Study II/ Figure 3A-B). These genes included key Th17-marker genes such as *IL17A*, *IL17F*, *IL23R* and *CCR6* while other important co-regulated genes included *FASL*, *NT5E*, *PRDM1* and *STAT4* (Study II/ Figure 3A-B). The Ingenuity pathway analysis identified pathways enriched in the genes affected by perturbation of FOSL. Enriched pathways include mitochondrial dysfunction, oxidative phosphorylation, T-helper cell differentiation, IL-23 signaling, interferon signaling, Th1/Th2/Th17 activation and autoimmune processes (RA and SLE). Many of these genes were validated using qRT-PCR, immunoblotting and flow cytometry analysis. Comparative analysis of FOSL target genes with STAT3 target genes including *RORγt* indicated an antagonistic relationship, as most genes positively regulated by STAT3 were negatively regulated by FOSL proteins. These findings imply that FOSL proteins negatively influence early stages of Th17 signaling in human.

### 5.2.3 FOSL1 and FOSL2 directly bind Th17-relevant genes

Genome wide occupancy profile of FOSL1 and FOSL2 was studied using ChIP-seq analysis. Overlap of FOSL binding motif at the target sites was observed. *De novo* motif enrichment analysis identified 22127 and 4088 binding sites for FOSL2 and FOSL1, respectively, with 3711 regions shared (Study II/ Figure 4A-D). Most binding sites were located in intronic or intergenic regions, suggesting FOSL may regulate gene expression through distal regulatory regions such as enhancer/silencer-mediated regulation. Interestingly, ChIP-seq and transcriptomic data revealed over 150 shared direct target genes, including Th17 relevant gene that were either activated (*IL7R*, *JAK2*, *IL13*, *BCL2A1*, *FASLG* and *PRDM1*) or repressed (*IL17F*, *IL23R*, *CXCR3* and *RORA*). FOSL binding overlap was observed over specific Th17 gene-targets (Study II/ Figure 4E). Subset of strongly co-regulated genes showed contrasting expression changes in FOSL DKD and DOE cells (Study II/ Figure 4E).

### 5.2.4 FOSL and BATF exhibit antagonistic regulation of Th17 genes

FOSL ChIP peaks showed the presence of binding motif for BATF, JUNB, FOS and ATF3 (Study II/ Figure 4C). While BATF is known to exhibit antagonistic relationship with FOSL2 in murine Th17 differentiation, role of BATF in human Th17 differentiation and if it regulates FOSL1 and FOSL2 responses in human Th17 cells was unknown. BATF expression was upregulated during human Th17 differentiation, and the transcriptomic analysis after BATF KD showed reduced expression of several genes including key Th17 marker genes such as *IL17A/F*, *IL23R*, *IL21* and *CCR6* (Study II/ Figure 5A-C). Comparative analysis of BATF KD against either DKD or DOE of FOSL showed that many Th17 lineage defining genes were regulated in the opposite manner by BATF and FOSL (Study II/ Figure 6A). BATF ChIP-seq identified genome wide occupancy over 16,000 sites in Th17 cells. The promoter regions of more than 4000 genes showed occupancy of BATF. Among the genes affected by BATF KD identified from RNAseq, 35 genes showed direct binding of BATF (Study II/ Figure 5E). Moreover, motif analysis of BATF ChIP-seq identified motif of other AP-1 factor including ATF3, JUNB, FOSL1 and FOSL2 were enriched within BATF peaks. Comparative analysis of genomic binding sites of BATF (16,479) and FOSL (FOSL1- 4058, FOSL2- 22,048) proteins revealed overlap of 2,624 sites (Study II/ Figure 6D). Importantly, several key Th17 genes were co-bound and regulated in opposing directions by these factors including *RORA, IL17F, IL23R, IL12RB2, FASLG* and *PRDM1*. Further analysis of BATF and FOSL proteins ChIP-seq peaks indicated that these factors occupy regulatory DNA elements, distal to promoter, that may alter the expression of their target genes. Such regulatory regions are known to show transcriptionally permissive histone modification like H3K27Ac marks (section 2.3.4). The shared binding sites of BATF, FOSL1 and FOSL2 at genes such as *IL17A, IL17F, IL23R, PRDM1* and *STAT4* showed flanking of H3K27Ac marks (reanalyzed from dataset GSE101389; Study II/ Figure 6E-F). These findings suggests that FOSL and BATF contextually or competitively bind to common set of genes at various regulatory regions for Th17 lineage specification.

Analysis of immunoprecipitation assays for FOSL1 and FOSL2 in our previous study (Shetty et al., 2021) revealed that FOSL proteins share 29 interactors, including JUN, JUNB, RUNX1, and SIRT1. BATF immunoprecipitation followed by immunoblot was performed to confirm common interactors such as JUN, JUNB, and RUNX1 (Study II/ Figure 7A-C). These results suggest that BATF and FOSL proteins potentially competes for common interactors for their functional antagonism in Th17 gene regulation.

### 5.2.5 Autoimmune disease associated SNPs disrupt FOSL and BATF binding

TFBS of FOSL1, FOSL2 and BATF were screened for presence of autoimmune associated SNPs based on our ChIP-seq data. The genome wide association studies (GWAS) catalogue from Caucasian population was used as primarily query SNPs linked with 11 different autoimmune diseases. Within the binding site of FOSL1, FOSL2 and BATF, we identified 114, 571 and 573 disease linked SNPs across 11 diseases (Study II/ Figure 8A). Moreover, 64 SNPs were shared between the three factors. SNPs overlapping with TFBS in the vicinity of Th17-related genes were shortlisted. DNA affinity precipitation assay was performed by designing oligonucleotides with the SNP (Mutant) and without (Wildtype) in the same binding site of each of the three factors (Study II/ Figure 8B). DAPA assays validated the selected SNPs in the binding site of FOSL1 near STAT3 and FOSL2 near TRAF3, BATF, CD28 and RUNX3. BATF binding for selected SNPs near STAT3, IL21E and GATA3 was also disrupted (Study II/ Figure 8C). Moreover, binding of all three factors was disrupted at the common site with SNPs near BCL10, SMAD3, LMOD3 and FRMD4B (Study II/ Figure 8D). These results suggests that SNPs associated with disease may affect the binding of these transcription factors.

## 5.3 A proximal enhancer regulates RORA expression during early human Th17 differentiation

### 5.3.1 Dynamic chromatin accessibility during Th17 differentiation

Chromatin accessibility is important feature for the regulation of gene expression in the cell differentiation. We performed ATAC-seq assay to reveal the dynamic chromatin accessibility of early differentiating Th17 cells. Samples were collected at 0, 2, 4, 24, 48 and 72 h of Th17 differentiation. The control samples were TCR activated without polarizing cytokines at each of those timepoints. Th17 differentiation was confirmed by expression of CCR6 cell surface marker and IL17A expression by TaqMan and ELISA assays (Study III/ Figure 1A-D). ATAC-seq data revealed approximately 20000 open chromatin regions in Th17 cells at all timepoints, with more peaks at later time points. Moreover, 1618-7278 loci were differentially accessible in Th17 cells at different time points, indicating lineage-specific remodeling of chromatin accessibility.

### 5.3.2 Th17 specific enhancers during of early differentiation

Specific histone modification such as H3K4me1, H3K4me3 and H3K27ac marks the regulatory region of DNA such as enhancers and promoters. To identify the enhancers in the Th17 cells, we performed ChIP-seq of those three histone modifications at 0.5 and 72 h of differentiation. ChromHMM machine learning tool revealed enhancers and promoters from the ChIP-seq data. Over 38000 enhancers were detected in Th17 cells at each timepoint. The majority of these enhancers were intronic followed by intergenic and promoter-overlapping enhancers. About one third of these enhancers had ATAC-seq peaks within them. At 0.5 h there were 154 and 184 enhancers specific to Th0 and Th17 cells, respectively while at 72 h the numbers were 2020 and 2473, respectively. By excluding previously reported Th1 and Th2 enhancers, we identified 66 and 1453 unique enhancers in Th17, while there were 6 and 792 enhancers for Th0 at 0.5 and 72 h respectively. The clusters of enhancers with high density of transcription factor binding sites are referred as super-enhancers (SE). Of the enhancers identified in Th17 cells, 4 and 130 were part of SE at 0.5 and 72 h respectively (Study III/ Figure 1F-G). Several enhancers overlapped with IBD patient biopsy-derived enhancers, suggesting their clinical relevance.

### 5.3.3 Th17 specific enhancers were associated with Th17 associated gene and TFs

We compared the Th17 specific enhancers to their neighboring genes differentially expressed in Th17 differentiating cells (compared to Th0 cells) identified in our previous study (Tuomela et al., 2016) to find out enhancer-gene associations. We identified 4 and 155 genes differentially expressed in the vicinity of these enhancers at 0.5 and 72 h, respectively. Interestingly, 140 out of 155 genes identified were upregulated during early stages of Th17 differentiation suggesting that the enhancers were primarily associated with upregulation of the transcription (Study III/ Figure 2).

Enrichment of TFBS motifs for the TF expressed in Th17 cells was tested within identified Th17 enhancers. There were 658 TFBS tested that identified 257 and 445 motifs enriched in enhancers at 0.5 and 72 h respectively. Motifs for key Th17 regulators such as RORγt, STAT3, and RORα were enriched only at 72 h, highlighting late-stage of lineage specification.

### 5.3.4 Th17 specific enhancers harbor autoimmune disease associated SNPs

Th17 specific enhancers were screened for the presence of autoimmune disease associated SNPs using snpEnrichR tool for 11 autoimmune diseases and three non-

autoimmune diseases. Several SNPs associated with autoimmune diseases such as MS, RA, SLE, T1D, Celiac disease, Ankylosing spondylitis, Crohn's disease, IgA deficiency, Primary biliary cholangitis, Polyendocrine syndrome and Ulceritis colitis were enriched in Th17 specific enhancers (72 h), while such enrichment was not found for SNPs associated with non-autoimmune diseases (Alzheimer's disease, Urinary incontinence and Age-related macular degeneration). This suggests that Th17 specific regulatory elements may specifically contribute to autoimmune disease susceptibility.

Presence of SNP in the TFBS can influence TF binding and affect transcription, and such SNPs are referred to as regulatory SNPs (rSNPs). We tested rSNPs present in the Th17 enhancers. A proximity criteria of 15bp was used to establish overlap of SNP and a TFBS. Total of 104 rSNPs across 42 enhancers were identified. Notably, a subset of these rSNPs based on TFBS analyzed from T cell specific ChIP-seq identified 16 enhancers with 23 rSNPs (Study III/ Table 2). Only 2 rSNPs were found in enhancers at 0.5h, while the remaining 21 were present in enhancers at 72 h. The most prevalent factors among them included NFATC1, BATF, TBET, GATA3, HIC1 and STATs.

The cis or trans expression quantitative trait loci (eQTL) effect on gene expression in blood was tested for all 104 rSNPs. The Th17 enhancers with 23 rSNPs had cis-eQTL effect on one or more of 48 genes. Only one rSNP had a trans-eQTL effect. Notably, 3 rSNPs strongly affected CTLA4 expression, linking enhancer regulation to autoimmunity (Study III/ Figure 3).

### 5.3.5 Super enhancer at RORA locus regulate Th17 cell differentiation

Among the top Th17 enhancers, two SE were present in the intron of RORA gene, while one SE was exclusively active in Th17 cells at 72 h. This SE contained five ATAC-seq peaks with strong H3K27ac/H3K4me1 enrichment. One of the ATAC-seq peaks overlapped with an enhancer region predicted to interact with RORA promoter based on GeneHancer database (Fishilevich et al., 2017). Additionally, the region overlapped with encode conserved cis-regulatory element (cCRE) (Study III/ Figure 4a). Luciferase assay was performed with four regions of the SE. The increased luciferase activity was confirmed with SE regions with a luciferase construct containing open enhancer regions while a control construct lacking those regions did not show increased activity (Study III/ Figure 4B-C).

Furthermore, a plasmid free *in vitro* assembled CRISPR-Cas9 protocol was utilized to delete the active region of SE. A 270-bp region containing TFBS for IRF4, MAF and STAT3 was selected for an *in vitro* guide RNA assembled Cas9 deletion. Naive T cells were nucleofected with two guide RNAs (L4 and R4) flanking this 270-bp

region referred to as a knockout (KO) along with a control (non-targeting and AAV1) guide RNA-Cas9. The deletion of the region was confirmed by PCR amplification, agarose gel electroporation and sanger sequencing of PCR amplified DNA. Importantly, deletion of the enhancer resulted in reduced RORA expression at RNA and protein level as well as decrease in IL-17A production functionally validating the enhancer's role in Th17 effector programming (Study III/ Figure 4D-G).

# 6 Discussion

## 6.1 Transcriptional remodeling of CD4+ T cells in the early stages of type 1 diabetes

Study I focused on understanding changes in CD4+ T cells during the early stages of progression of T1D in young children. Our study utilized over 90000 high quality single cells to identify 10 cell populations within CD4+ T cells: three populations of naive T cells, two populations of central memory cells, three populations of helper T cells and two populations of regulatory T cell phenotypes. While cell type proportions between cases and controls remained unchanged, significant DE genes were found in naive and central memory T cell subsets. Many genes were affected in effector cells in those who later progressed to T1D. Several pathways including viral and interferon-related pathways were enriched in children progressing to T1D. Our study also identified a gene regulatory network composed of regulons that regulate transcriptional state of these cell subsets. The differential activity of regulons such as PRDM1 and GATA3 was identified in specific cell subsets.

Previous bulk transcriptomic and flow cytometry studies have reported altered cell population frequencies of regulatory T cells or effector T cells in individuals at risk for T1D (Hull, Peakman and Tree, 2017). Our results suggest that early immune dysregulation in CD4+ T cells may be more accurately reflected in transcriptional programming within specific subsets rather than in changes in cell numbers. This underscores the critical role of single-cell approaches in detecting subtle, yet functionally important, changes that bulk analyses might overlook.

Our study identified many genes associated with the case-control status or age within CD4+ T cell subsets. Among the disease-associated genes, *CD69*, *PLGRKT*, and *CD226* have also been implicated in recent T1D GWAS highlighting the potential importance of these genes in the disease (Robertson et al., 2021). Five of the eight genes (*PSME4, NTPCR, SLC35E4, MFHAS1* and *IQGAP2*) significantly differentially expressed between the cases and controls, had been previously associated with T1D or related disorders further underscoring their potential relevance. For instance, the differential expression of *PSME4* and *NTPCR* in cases aligns with findings from previous bulk transcriptomic studies (Bediaga et al., 2022; Kallionpaa et al., 2019), while the hypermethylation of the SLC35E4 promoter

region in children progressing to T1D before seroconversion suggests epigenetic regulation to be involved in in disease progression (Starskaia et al., 2022). IQGAP2 has been shown to interact with glycogen synthesis regulators and to affect the phosphorylation of components of insulin pathway in liver in mice. Deletion of *Iqgap2* resulted in increased plasma insulin levels (Sen et al., 2023). Upregulation of *MFHAS1* and *IQGAP2* may reflect heightened immune activation. Notably, these changes were most pronounced in naive and central memory subsets, suggesting that developmental trajectories of these cells may be altered in children progressing to autoimmunity.

The enrichment of pathways such as the "interferon-mediated signaling pathway" and "response to virus" in multiple T cell subsets is consistent with existing literature linking interferon and viral responses to T1D pathogenesis (Coppieters et al., 2012; Ferreira et al., 2014; Kallionpaa et al., 2014; Lernmark et al., 2025). The enrichment of cell type-specific terms like "interferon beta production" and "interleukin-10 production" in naive and effector Tregs, respectively, highlights the intricate role of these cytokines in Tregs proliferation and immunosuppressive functions. These findings suggest that immune activation, particularly in naive and central memory subsets, may be a critical early event in the development of autoimmunity in T1D.

Our study also elucidated cell-type-specific gene regulatory networks, or regulons that govern the transcriptional state of CD4+ T cell subsets. The differential activity of regulons, particularly the upregulation of PRDM1 in Th17 cells and the downregulation of GATA3 in naive T cells, offers insights into the regulatory mechanisms underlying T1D. Notably, Prdm1(Blimp1) regulates T cell activation and prevents inflammation by repressing *Il17a* locus and upregulating FOXP3 expression (Ogawa et al., 2018). In the Study II, we reported that PRDM1 was in the protein complex with FOSL proteins or BATF and act as the negative regulators of Th17 differentiation. Jain *et al.,* reported that murine *Blimp1* deletion in Th17 cells reduced its activation as well as EAE development. Blimp1-mediated functions in Th17 cells in turn are dependent on RORC (Jain et al., 2016). These observations highlight the context-dependent interplay between PRDM1 and RORC in regulating human and murine Th17 cell differentiation by modulating gene expression program of CD4+ T cells. The increased PRDM1 regulon activity observed in Th17 cells of children progressing to the disease may reflect a compensatory regulatory response during the early stages of disease. However, the persistence of autoimmunity despite this upregulation suggests that such mechanisms are either insufficient or overridden by pro-inflammatory signals. Together, these findings highlight candidate biomarkers and pathways that may serve as early indicators of disease risk.

The strength of our study lies in the use of a unique, homogeneous longitudinal birth cohort of children who progressed to seroconversion before the age of two and

developed T1D before the age of five. This longitudinal sampling provided valuable temporal resolution, allowing us to identify immune changes early on from birth to disease initiation. Our use of scRNA-seq to resolve the granularity of CD4+ T cell compartment within each of the naive, memory, effector and regulatory T cell phenotypes offers a detailed perspective on the immune dysregulation associated with T1D.

Concerning the study's limitations, it should be noted that T1D is a heterogeneous disease with varying rate of disease progression, distinct first appearance of autoantibodies (IAA, GADA) and variability in the age at disease diagnosis. These features may affect early immune modulation in the initiation of autoimmunity, which has not been addressed in the present study.

Additionally, the modest cohort size may limit the generalizability of our findings. Transcriptional changes identified were not validated at the protein or functional level. Here we focused solely on the CD4+ T cell compartment to resolve their cellular granularity and such single cell studies on other immune cells involved in T1D, such as CD8+ T cells, B cells, and innate immune cells are needed. Our analysis is restricted to peripheral blood and is likely to capture at least partly immune events different from those occurring within pancreatic islets.

To build upon our findings, future research should expand cohort size and diversity to validate our observations across different genetic and environmental backgrounds. Multi-omic approaches, including TCR clonotype sequencing, single-cell ATAC-seq, and proteomics, could offer complementary insights into the regulatory mechanisms at play. Future studies should incorporate pancreatic islet samples to provide a more comprehensive understanding of T1D pathogenesis. These will be difficult to access at the stage before seroconversion and from the type of individuals we have studied here. By correlating identified immune changes with their functional impact on beta cell destruction, we can gain a more holistic understanding of T1D. Ultimately, these efforts may lead to the identification of candidate biomarkers and pathways that serve as early indicators of disease risk, paving the way for targeted interventions in T1D.

## 6.2 Transcriptional regulation by FOSL1, FOSL2 and BATF during CD4+ T cell differentiation towards Th17

Study II provides insights into the transcriptional regulation of early human Th17 differentiation, emphasizing the roles of the AP-1 family members FOSL1 and FOSL2 as negative regulators. Th17 polarization is facilitated by cytokines such as IL-6 and IL-1β, which enhance STAT3 activity, a key driver of Th17 lineage commitment. Our findings reveal that these cytokines not only promote STAT3

activity but also upregulate FOSL1 and FOSL2, suggesting a complex regulatory landscape where STAT3-mediated initiation of Th17 differentiation is also controlled by FOSL proteins to limit lineage commitment when relevant.

The discovery that FOSL2 is directly regulated by STAT3 introduces a potential feedback mechanism within the Th17 differentiation pathway. This feedback loop implies that while STAT3 is crucial for initiating Th17 differentiation, it concurrently induces the expression of FOSL proteins, which act as a brake on this process. This regulatory balance is essential for maintaining immune homeostasis and preventing the overproduction of Th17 cells, which are implicated in various autoimmune diseases.

Our study corroborates the role of BATF as a positive regulator of human Th17 differentiation, consistent with previous findings (section 2.3.3). BATF operates synergistically with IRF4 and STAT3 to promote Th17-associated gene expression (Li et al., 2012). Importantly, we report a functional antagonism between BATF and FOSL proteins, highlighting a competitive interplay at the genomic level. FOSL proteins co-localize with BATF at multiple genomic sites, including intergenic, intronic, and promoter regions of key Th17 genes, exerting opposing regulatory effects. This antagonism likely arises from competitive binding dynamics and the differential recruitment of co-factors, underscoring the complexity of transcriptional regulation in Th17 lineage specification.

The contextual binding of FOSL and BATF at key regulatory sites suggests that Th17 lineage specification is not solely dictated by the presence of TFs but also by their relative abundance, binding affinity, and protein-protein interactions. This intricate regulatory network may account for the observed heterogeneity within Th17 populations, where subsets display variable effector potential. Such heterogeneity is crucial for the diverse functional roles of Th17 cells in immune responses, ranging from protective immunity to pathogenic autoimmunity.

Our study also establishes a direct link between mechanistic findings and human disease through the identification of autoimmune disease-associated SNPs within FOSL and BATF binding sites. These SNPs could disrupt transcription factor binding, tilting the balance between FOSL-mediated repression and BATF-mediated activation, potentially skewing Th17 responses toward pathogenicity. This observation supports the hypothesis that genetic variation in regulatory elements, rather than coding sequences, contributes to autoimmune susceptibility by altering transcriptional networks. Our findings provide a framework for understanding how SNPs can modulate immune cell fate decisions, offering new avenues for therapeutic intervention.

Interestingly, while our study identifies FOSL1 and FOSL2 as negative regulators of human Th17 lineage, previous murine studies have reported FOSL1 as a positive regulator (Moon et al., 2017). This discrepancy highlights the importance

of species-specific differences in transcriptional regulation. However, the role of FOSL2 as a repressor in murine Th17 lineage (Section 2.3.3, Ciofani et al., 2012) aligns with our findings, and is an example of conserved mechanisms across species. Our study is pioneering in elucidating the transcriptional interactions among these factors in human Th17 cells, providing a foundation for future research.

The FOSL–STAT3–BATF axis may represent a general principle of immune regulation, where lineage-promoting signals are counteracted by lineage-restraining factors to maintain immune homeostasis. Targeting FOSL proteins or BATF could offer novel strategies to modulate Th17 responses in autoimmune diseases. Enhancing FOSL activity might suppress pathogenic Th17 cells, while inhibiting FOSL function could boost protective Th17 responses in infection.

Further studies should investigate the dynamics and precise modes of action of FOSL and BATF binding *in vivo*, the role of co-factors in determining transcriptional outcomes, and the impact of disease-associated SNPs on chromatin accessibility and transcription factor recruitment. FOSL and BATF bound sites largely co-localize within intronic elements that could be part of enhancer regions. While gene enhancers are well known to harbor TFBS and govern lineage identity and plasticity of helper T cells, the differentiation induced epigenomic changes directed by AP-1 transcription factors such as FOSL and BATF can be addressed in future studies. Integrating single-cell transcriptomics and epigenomics will be critical to dissect the heterogeneity of Th17 populations under FOSL regulation.

## 6.3 Gene enhancer landscape regulates CD4+ T cell differentiation towards Th17

Study III provides a comprehensive analysis of the enhancer landscape during Th17 cell differentiation by utilizing genomic technologies such as ChIP-seq and ATAC-seq. By integrating these methodologies, we were able to capture the dynamic chromatin accessibility and histone modification patterns that characterize the differentiation process of Th17 cells. Our findings underscore the importance of epigenetic regulation in T helper cell lineage specification and offer new insights into the molecular underpinnings of Th17 differentiation.

One of the key revelations from our study is the identification of thousands of unique open chromatin regions that emerge at various time points during Th17 differentiation, from as early as 2-4 h to 72 h. This temporal aspect of chromatin accessibility suggests a staged process of enhancer activation that likely reflects the sequential engagement of transcriptional programs required for Th17 lineage commitment. The integration of ATAC-seq data with histone modification profiles allowed us to pinpoint enhancers that are active during Th17 specification and link them to nearby genes. Notably, these Th17 specific enhancers are associated with

the upregulation of several key lineage-defining TFs such as STAT3, RORγt and RORα, highlighting their crucial role in orchestrating the differentiation process.

Our motif enrichment analysis provides further mechanistic insights, revealing that early-stage enhancers lack motifs for key lineage defining TFs. This observation suggests that initial chromatin remodeling events may serve as a priming mechanism, setting the stage for subsequent TF binding and the activation of Th17 gene programs. This priming effect underscores the complexity of transcriptional regulation, where chromatin accessibility and TF availability are finely tuned to ensure precise gene expression patterns.

Another significant aspect of our study is the identification of autoimmune disease–linked single nucleotide polymorphisms (SNPs) within Th17 enhancers. The enrichment of these SNPs in enhancers active at 72 h indicates that disease-associated variants potentially contribute to lineage specification at this stage. Many of these SNPs overlap with TF binding sites, suggesting that they may disrupt enhancer activity, alter TF binding, and consequently influence gene expression. Our eQTL analysis confirmed that these SNPs influence the expression of genes with established roles in immune regulation, thereby providing a potential mechanistic link between genetic variation and autoimmune pathogenesis.

RORα, together with the lineage-defining factor RORγt, contributes to the stability of the Th17 effector program (Hall et al., 2022; Yang et al., 2008). Pharmacological inhibition of RORA has been shown to effectively suppress Th17-driven inflammatory disorders (Wang et al., 2021). The functional validation of a Th17 specific super-enhancer at the RORA locus further underscores the significance of enhancer-mediated control of key Th17 TFs. The presence of key TF binding sites, such as those for AP-1 factors and STAT3, within this enhancer highlights the cooperative regulation by multiple TFs in driving Th17 differentiation. This finding aligns with the concept of enhancer hubs, where multiple TFs converge to regulate critical gene expression, thereby ensuring robust lineage commitment.

Our study also suggests that extensive chromatin remodeling at regulatory enhancers is a response to cytokine and TCR signaling during Th17 differentiation, thereby facilitating transcriptional reprogramming. This dynamic interplay between signaling pathways and chromatin architecture emphasizes the plasticity of the epigenome in adapting to external cues and directing cell fate decisions (Renaude et al., 2020).

While our study provides important insights into enhancer remodeling during Th17 differentiation, several limitations should be acknowledged. First, the reliance on *in vitro* differentiation systems may not fully capture the complexity of *in vivo* immune environments. Studies in patient samples or animal models would be valuable to complement these findings and clarify their physiological relevance. Second, although we identified thousands of candidate enhancers and many

disease-associated SNPs, functional validation was limited to the RORA super-enhancer, leaving the majority of regulatory elements untested. Third, the temporal resolution of our analyses, while informative, may overlook finer dynamics of enhancer activation that could be revealed through single-cell multi-omic approaches. Finally, the impact of autoimmune-associated SNPs was inferred from enrichment and eQTL analyses rather than direct allele-specific functional assays. Future studies employing CRISPR-based enhancer perturbation, DNA binding assays, and single-cell profiling will be useful to dissect the causal roles of these enhancers and variants. Such approaches will not only refine our understanding of Th17 lineage specification but also open translational opportunities to target enhancer hubs or SNP-disrupted regulatory elements as novel therapeutic strategies in autoimmune disease.

# 7 Conclusions

This thesis focused on studying transcriptional rewiring of CD4+ T cells in context of autoimmune diseases. The aim of the work presented in this dissertation was to characterize transcriptomic changes in CD4+ T cells during the progression of autoimmune T1D and provide a multilayer understanding of the transcriptional and epigenomic programs that drive $CD4^+$ T cell polarization toward the Th17 lineage. The key methods used in the studies were genome wide sequencing technologies for unbiased discoveries in each of the studies as follows:

Study I demonstrate that early T1D progression is characterized by transcriptional and regulatory rewiring of CD4+ T cell populations and include processes characteristic for antiviral and interferon responses. These changes destabilize tolerance networks and prime effector differentiation, providing mechanistic insight into how environmental triggers interact with immune development to initiate autoimmunity.

Study II identifies FOSL1 and FOSL2 as critical negative regulators of Th17 differentiation, acting in antagonism with BATF. FOSL2 functions downstream of STAT3 signaling. This FOSL1 and FOSL2 mediated gene regulatory network not only refines our understanding of Th17 biology but also provides mechanistic insights into genetic variations at their TFBS that may contribute to autoimmune pathogenesis. By highlighting the balance between positive regulator BATF and negative regulators FOSLs, these findings underscore the complexity of Th17 lineage specification and suggests new avenues for therapeutic intervention.

Study III maps the dynamic enhancer landscape during early Th17 differentiation and revealed chromatin accessibility at enhancer regions of the key Th17 loci. Autoimmune disease–associated SNPs were enriched in Th17 enhancers providing a link between genetic variation and immune dysregulation. Th17 specific super-enhancer at the RORA locus highlights enhancer-mediated control of lineage-defining transcription factors. In summary, our study reports the novel dynamic enhancer landscape during early human Th17 differentiation, provides new mechanistic insights into its role in Th17 specification and a framework for future studies of regulatory elements in autoimmune diseases.

# Acknowledgements

I would like to express my deepest gratitude to my PhD supervisor, Riitta Lahesmaa. Your expertise, motivation, and enthusiasm for high-quality research have guided and inspired me throughout this journey. Your encouragement and patience shaped every stage of this work. I feel fortunate to have carried out my doctoral studies in your laboratory and am sincerely grateful for the many opportunities you have provided. Thank you for your trust and mentorship.

I am also deeply grateful to my PhD co-supervisor, Ubaid Ullah Kalim. Your expertise and guidance have been invaluable in helping me progress in my research. Thank you for generously sharing your knowledge and experience. I have learned a great deal from working with you, and I appreciate the encouragement that supported my scientific growth.

My sincere thanks go to the pre-examiners of this thesis, Kirsi Rautajoki and Minna Kaikkonen-Määttä, for taking the time to carefully review my work and for providing insightful suggestions that strengthened the thesis. I would also like to thank Lucy Walker for accepting the invitation to serve as my opponent. I look forward to our discussion during the dissertation.

I am grateful to my thesis follow-up committee members, Laura Elo and Tapio Lönnberg, who have also been among my most important collaborators. Laura, your expertise and that of your bioinformatics research group—made these studies possible. Tapio, thank you for helping me with single-cell sample processing and for your valuable feedback and expertise. I appreciate your active collaboration and timely support throughout this project.

My sincere thanks to Mikael Knip for providing the invaluable samples for Study I and for your expertise in completing the work.

I also thank all co-authors of the research studies included in this thesis. Your contributions made it possible to complete the projects and publish the resulting work.

I am grateful to the Turku Doctoral Programme of Molecular Medicine (TuDMM) and the InFLAMES Doctoral Module for providing excellent doctoral training courses and educational events. The student activities and annual gatherings offered meaningful opportunities to connect with peers and share our PhD

experiences. The InFLAMES Flagship enabled me to engage with the local immunology community and learn about the impactful research conducted in Turku. I am especially thankful for the opportunities to meet world-class immunologists visiting the flagship and to learn from their expertise. I also thank both InFLAMES and TuDMM for their financial support, which enabled me to carry out my research and to participate in numerous scientific conferences where I could present my work to the broader scientific community.

Next, I would like to thank my colleagues in the Molecular Systems Immunology group, both past and present lab members: Ankitha, Tanja, Santosh, Essi, Lea, Emilie, Toni, Moin, Meraj, Julius, Roosa P., Jimmy, Omid, Bilal, Ubaid, Sarita, Marjo, Robert, Kedar, Olof, Inna, Nikita, Roosa K., Karolina, Upasana, Gopika, Amogh, Saumya, and Ayush. It has been a pleasure to work with you and to learn from each of you. I am grateful for the many activities we enjoyed together over the years—Christmas Café, mölkky games, after-work gatherings, summer retreats, science days, and many more. You have all contributed to creating a cheerful and supportive environment for everyday work. Special thanks to Ankitha for helping me with everything from traveling to Finland to settling in and meeting new friends. You have been a wonderful friend, colleague and mentor in the lab, and I am glad I could contribute to Study II led by you. You are an inspiring scientist. I also thank Tanja for always being there when I needed help and for offering valuable suggestions for experiments. My thanks to Kedar and Robert for our morning coffee sessions and many interesting discussions on diverse topics covering science, history, politics, running and beyond. I am grateful to Omid for being the backbone of all practical matters in the lab and for always being available with help and advice. Thank you, Anne, for helping with all the practical office matter. My sincere thanks to Marjo and Sarita for your essential support in the lab—you have been crucial to the success of all the projects.

This PhD dissertation was conducted at the Turku Bioscience Centre, University of Turku and Åbo Akademi University. I owe special appreciation to the excellent support at the Turku Bioscience Centre throughout these years. The monthly assembly meetings have been a joyful place to meet colleagues and learn about the frontier research conducted at our centre. The core facilities including the Single-Cell Core, the Finnish Functional Genomics Centre (FFGC), the Cytometry Core Unit, and others have made the studies in this thesis possible. My heartfelt thanks to all core facility personnel who keep these units running smoothly. I especially acknowledge Paivi and Oso from FFGC for their help with Study I project. I appreciate help from Sirkku, Katri, Henok and Maija on various matters.

I am also grateful for the leadership opportunities offered through the PhD–Postdoc Board at Turku Bioscience and for the colleagues who have helped build this early-career researcher network to support each other. I am greatful for my

colleageues and friends at young Scandivanian Society for Immunology (ySSI)-Martin, Kenneth, Helka, Markus and others. We have truly been able to build and be part of the early career researchers network in Nordics and connect with young European immunologist network. It was fun to organize many events with your support. It has been a valuable community for learning, collaboration, and growth.

I have been fortunate to experience the friendship of many wonderful people. My friends from the TMA group—many of you, I met during my very first week in Finland: Jismi, Shruti, Mayank, Madhukar, Afshan, Sachin, Ashwini, Sibi, Joakim, Ekta, Anand, and Omkar—have been an important part of this journey. Many of us have shared the PhD path together and are now seeing it reach completion. The laughter, delicious food, fun moments, and gazillions of activities, events, and celebrations over the past years have brought so much joy to my life in Finland. Ashwini, I am truly lucky to have you as a friend and classmate from our bachelor's and master's days at IISER Pune to now completing our PhDs at the same university. Thank you, Joakim, for hosting me at Vaasa over Midsummers and new year celebrations. I am grateful that our friendship has grown into a family away from home. I am also thankful for my Marathi group of friends—Kedar, Renuka, Santosh, Rahul Yewale, Sachin, and Ashwini—for all our festive celebrations, good times, and shared meals. Kedar, Renuka, and little Aarvi, thank you for always welcoming me into your family and giving me a sense of home. Santosh, Preeti, and little Veda, thank you for welcoming me into your family just across the sea in Denmark and beyond in recent years. My heartfelt thanks to Pathiban, Kalai, and little Ameresh for welcoming me into your family, for all the good times, tennis lessons, and road trips we shared in Finland. Thank you to Shiyana, Nikhil, and little Kian—I am lucky to have your family as my upstairs neighbours. I would also like to thank the Indian community in Turku for the Diwali celebrations we enjoyed together.

I am also grateful for my Cryparty friends group: Sara, Ines (and Archie), Diogo, Emilie, Amna, Barbara, Iman, Blanca, and Giulia. We shared our PhD journeys in the same building, BioCity, and its research facilities. I am proud that despite the challenges of the Covid years, we found each other, supported one another, and built strong friendships. Thank you for all the fun times, games, and birthday celebrations. Special thanks to Sara and Ines for hosting so many of these gatherings.

My thanks also go to Pinja, Roosa, Eetu, Mira, Eelis, Tanja, and Jalmari for your close friendship over the past couple of years and for all the good times we shared. Thank you, Magnus and Maria, for welcoming me into your family. I would like to thank Matias for your friendship. It was fun to organize events together at the Haliskylä tenants committee.

I owe my warmest thanks to my Indian friends who are always just a call away and who spend time with me whenever I am home in India. My high school friends—Saurabh, Sachin R, Prem, Arun, Dodal, and Shantanu—have been a constant source

of support. Thank you, Sanskruti, for always being there and for following your own path to Europe for studies and work—and for visiting me in Turku.

My friends from IISER Pune—Kabra, Gokul, Chary, Kaustav, and Sumit—have continued to be an important part of my life. Thank you, Kabra, for always being just a call away and for our long conversations trying to unravel life together. Thank you, Kaustav, for being an inspiring friend and for visiting me in Turku. I also thank my Master's thesis mentor and dear friend, Satyajeet Khare, for hosting me many times at your institute in Pune and for your constant mentorship.

I am deeply fortunate to have a supportive family back home who have been my constant cheerleaders—my parents, Aai and Aapa; my siblings and their partners, Tai (Suwarna) and Bhauji (Shivkumar), Bhaiya (Rohit) and Priyanka; and my nephew and nieces, Naksh, Ruhi, little Riya, and the youngest, Diyvanka. Thank you all for your endless love, support, and understanding. Love you all!

Turku, May 2026
*Rahul Biradar*

# References


Acerbi, E., Viganò, E., Poidinger, M., Mortellaro, A., Zelante, T., & Stella, F. (2016). Continuous time Bayesian networks identify Prdm1 as a negative regulator of TH17 cell differentiation in humans. Scientific reports, 6(1), 23128. https://doi.org/10.1038/srep23128

Acosta-Rodriguez, E. V., Napolitani, G., Lanzavecchia, A., & Sallusto, F. (2007). Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17–producing human T helper cells. Nature immunology, 8(9), 942-949. https://doi.org/10.1038/ni1496

Acuto, O., & Michel, F. (2003). CD28-mediated co-stimulation: a quantitative support for TCR signalling. Nature Reviews Immunology, 3(12), 939-951.

Aggarwal, S., Ghilardi, N., Xie, M. H., de Sauvage, F. J., & Gurney, A. L. (2003). Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. Journal of Biological Chemistry, 278(3), 1910-1914. https://doi.org/10.1074/jbc.M207577200

Ahlfors, H., Limaye, A., Elo, L. L., Tuomela, S., Burute, M., Gottimukkala, K. V. P., ... & Lahesmaa, R. (2010). SATB1 dictates expression of multiple genes including IL-5 involved in human T helper cell differentiation. Blood, The Journal of the American Society of Hematology, 116(9), 1443-1453. https://doi.org/10.1182/blood-2009-11-252205

Ahmad, R., Narwaria, M., Singh, A., Kumar, S., & Haque, M. (2023). Detecting diabetic ketoacidosis with infection: combating a life-threatening emergency with practical diagnostic tools. Diagnostics, 13(14), 2441.

Akimzhanov, A. M., Yang, X. O., & Dong, C. (2007). Chromatin remodeling of interleukin-17 (IL-17)-IL-17F cytokine gene locus during inflammatory helper T cell differentiation. Journal of Biological Chemistry, 282(9), 5969-5972. https://doi.org/10.1074/jbc.C600322200

Alleva, D. G., Pavlovich, R. P., Grant, C., Kaser, S. B., & Beller, D. I. (2000). Aberrant macrophage cytokine production is a conserved feature among autoimmune-prone mouse strains: elevated interleukin (IL)-12 and an imbalance in tumor necrosis factor-alpha and IL-10 define a unique cytokine profile in macrophages from young nonobese diabetic mice. Diabetes, 49(7), 1106-1115. https://doi.org/10.2337/diabetes.49.7.1106

Anderson III, D. A., Dutertre, C. A., Ginhoux, F., & Murphy, K. M. (2021). Genetic models of human and mouse dendritic cell development and function. Nature Reviews Immunology, 21(2), 101-115. https://doi.org/10.1038/s41577-020-00413-x

Arif, S., Moore, F., Marks, K., Bouckenooghe, T., Dayan, C. M., Planas, R., ... & Peakman, M. (2011). Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated β-cell death. Diabetes, 60(8), 2112-2119.

Arif, S., Tree, T. I., Astill, T. P., Tremble, J. M., Bishop, A. J., Dayan, C. M., ... & Peakman, M. (2004). Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. The Journal of clinical investigation, 113(3), 451-463.

Ashby, K.M., Hogquist, K.A. A guide to thymic selection of T cells. Nat Rev Immunol 24, 103–117 (2024). https://doi.org/10.1038/s41577-023-00911-8

Babon, J. A. B., DeNicola, M. E., Blodgett, D. M., Crèvecoeur, I., Buttrick, T. S., Maehr, R., ... & Kent, S. C. (2016). Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nature medicine, 22(12), 1482-1487.

Banting, F. G., Best, C. H., Collip, J. B., & Macleod, J. (1922). Preliminary studies of the physiological of insulin. Proc. Trans. R. Soc. Canada, 16, 18.

Basdeo, S. A., Cluxton, D., Sulaimani, J., Moran, B., Canavan, M., Orr, C., ... & Fletcher, J. M. (2017). Ex-Th17 (nonclassical Th1) cells are functionally distinct from classical Th1 and Th17 cells and are not constrained by regulatory T cells. The Journal of Immunology, 198(6), 2249-2259. https://doi.org/10.4049/jimmunol.1600737

Bedford, J. G., Heinlein, M., Garnham, A. L., Nguyen, T. H., Loudovaris, T., Ge, C., ... & Wakim, L. M. (2020). Unresponsiveness to inhaled antigen is governed by conventional dendritic cells and overridden during infection by monocytes. Science Immunology, 5(52), eabb5439. DOI: 10.1126/sciimmunol.abb5439

Bediaga, N. G., Garnham, A. L., Naselli, G., Bandala-Sanchez, E., Stone, N. L., Cobb, J., ... & Harrison, L. C. (2022). Cytotoxicity-related gene expression and chromatin accessibility define a subset of CD4+ T cells that mark progression to type 1 diabetes. Diabetes, 71(3), 566-577. https://doi.org/10.2337/db21-0612

Behmoaras, J., Mulder, K., Ginhoux, F., & Petretto, E. (2025). The spatial and temporal activation of macrophages during fibrosis. Nature Reviews Immunology, 1-15. https://doi.org/10.1038/s41577-025-01186-x

Bell, K. J., & Lain, S. J. (2025). The Changing Epidemiology of Type 1 Diabetes: A Global Perspective. Diabetes, obesity & metabolism, 27 Suppl 6(Suppl 6), 3–14. https://doi.org/10.1111/dom.16501

Bender, C., Rodriguez-Calvo, T., Amirian, N., Coppieters, K. T., & von Herrath, M. G. (2020). The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes. Science advances, 6(42), eabc5586. DOI: 10.1126/sciadv.abc5586

Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., ... & Ochs, H. D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nature genetics, 27(1), 20-21.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., ... & Kuchroo, V. K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature, 441(7090), 235-238. https://doi.org/10.1038/nature04753

Biradar, R., Kalim, U. U., Lönnberg, T., Junttila, S., Suomi, T., Norman, S. Z., ... & Lahesmaa, R. (2025). Single-cell RNA-seq analysis of longitudinal CD4+ T cell samples reveals cell-type-specific changes during early stages of type 1 diabetes. Genome medicine, 17(1), 154. https://doi.org/10.1186/s13073-025-01574-x

Bonifacio, E., & Ziegler, A. G. (2010). Advances in the prediction and natural history of type 1 diabetes. Endocrinology and Metabolism Clinics, 39(3), 513-525. https://doi.org/10.1016/j.ecl.2010.05.007

Bonilla, F. A., & Oettgen, H. C. (2010). Adaptive immunity. Journal of Allergy and Clinical Immunology, 125(2), S33-S40.

Bouchery, T., Kyle, R., Ronchese, F., & Le Gros, G. (2014). The differentiation of CD4+ T-helper cell subsets in the context of helminth parasite infection. Frontiers in immunology, 5, 487.

Bour-Jordan, H., & Bluestone, J. A. (2007). B cell depletion: a novel therapy for autoimmune diabetes?. The Journal of clinical investigation, 117(12), 3642-3645.

Brunkow, M. E., Jeffery, E. W., Hjerrild, K. A., Paeper, B., Clark, L. B., Yasayko, S. A., ... & Ramsdell, F. (2001). Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nature genetics, 27(1), 68-73.

Brüstle, A., Heink, S., Huber, M., Rosenplänter, C., Stadelmann, C., Yu, P., ... & Lohoff, M. (2007). The development of inflammatory TH-17 cells requires interferon-regulatory factor 4. Nature immunology, 8(9), 958-966. https://doi.org/10.1038/ni1500

Buchacher, T., Shetty, A., Koskela, S. A., Smolander, J., Kaukonen, R., Sousa, A. G., ... & Lahesmaa, R. (2023). PIM kinases regulate early human Th17 cell differentiation. Cell Reports, 42(12). https://doi.org/10.1016/j.celrep.2023.113469

Capone, A., & Volpe, E. (2020). Transcriptional regulators of T helper 17 cell differentiation in health and autoimmune diseases. Frontiers in immunology, 11, 348.

Capone, A., Naro, C., Bianco, M., De Bardi, M., Noël, F., Macchi, P., ... & Sette, C. (2021). Systems analysis of human T helper17 cell differentiation uncovers distinct time-regulated transcriptional modules. Iscience, 24(5). https://doi.org/10.1016/j.isci.2021.102492

Carlos, D., Costa, F. R., Pereira, C. A., Rocha, F. A., Yaochite, J. N., Oliveira, G. G., ... & Silva, J. S. (2017). Mitochondrial DNA activates the NLRP3 inflammasome and predisposes to type 1 diabetes in murine model. Frontiers in Immunology, 8, 164. https://doi.org/10.3389/fimmu.2017.00164

Carr, T. M., Wheaton, J. D., Houtz, G. M., & Ciofani, M. (2017). JunB promotes Th17 cell identity and restrains alternative CD4+ T-cell programs during inflammation. *Nature communications*, *8*(1), 301. https://doi.org/10.1038/s41467-017-00380-3

Carrero, J. A., McCarthy, D. P., Ferris, S. T., Wan, X., Hu, H., Zinselmeyer, B. H., ... & Unanue, E. R. (2017). Resident macrophages of pancreatic islets have a seminal role in the initiation of autoimmune diabetes of NOD mice. Proceedings of the National Academy of Sciences, 114(48), E10418-E10427. https://doi.org/10.1073/pnas.1713543114

Castro, G., Liu, X., Ngo, K., De Leon-Tabaldo, A., Zhao, S., Luna-Roman, R., ... & Fung-Leung, W. P. (2017). RORγt and RORα signature genes in human Th17 cells. PloS one, 12(8), e0181868. https://doi.org/10.1371/journal.pone.0181868

Cerboni, S., Gehrmann, U., Preite, S., & Mitra, S. (2021). Cytokine-regulated Th17 plasticity in human health and diseases. Immunology, 163(1), 3-18.

Chen, S., Zhu, H., & Jounaidi, Y. (2024). Comprehensive snapshots of natural killer cells functions, signaling, molecular mechanisms and clinical utilization. Signal Transduction and Targeted Therapy, 9(1), 302.

Cho, J., Pietras, E., Garcia, N., Ramos, R. I., Farzam, D., Monroe, H., ... & Miller, L. (2010). IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection (37.50). The Journal of Immunology, 184(Supplement_1), 37-50.

Ciofani, M., Madar, A., Galan, C., Sellars, M., Mace, K., Pauli, F., ... & Littman, D. R. (2012). A validated regulatory network for Th17 cell specification. Cell, 151(2), 289-303. 10.1016/j.cell.2012.09.016

Coppieters, K. T., Boettler, T., & von Herrath, M. (2012). Virus infections in type 1 diabetes. Cold Spring Harbor perspectives in medicine, 2(1), a007682. https://doi.org/10.1101/cshperspect.a007682

Cretney, E., Xin, A., Shi, W., Minnich, M., Masson, F., Miasari, M., ... & Kallies, A. (2011). The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. Nature immunology, 12(4), 304-311. 10.1038/ni.2006

Crotty, S. (2014). T follicular helper cell differentiation, function, and roles in disease. Immunity, 41(4), 529-542.

Crotty, S. (2019). T follicular helper cell biology: a decade of discovery and diseases. Immunity, 50(5), 1132-1148.

Crotty, S. (2021). Revealing T follicular helper cells with BCL6. Nature Reviews Immunology, 21(10), 616-617.

Cua, D. J., Sherlock, J., Chen, Y. I., Murphy, C. A., Joyce, B., Seymour, B., ... & Sedgwick, J. D. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature, 421(6924), 744-748. https://doi.org/10.1038/nature01355

Culina, S., Lalanne, A. I., Afonso, G., Cerosaletti, K., Pinto, S., Sebastiani, G., ... & ImMaDiab Study Group. (2018). Islet-reactive CD8+ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. Science immunology, 3(20), eaao4013.

Damond, N., Engler, S., Zanotelli, V. R., Schapiro, D., Wasserfall, C. H., Kusmartseva, I., ... & Bodenmiller, B. (2019). A map of human type 1 diabetes progression by imaging mass cytometry. Cell metabolism, 29(3), 755-768. https://doi.org/10.1016/j.cmet.2018.11.014

Dardalhon, V., Awasthi, A., Kwon, H., Galileos, G., Gao, W., Sobel, R. A., ... & Kuchroo, V. K. (2008). IL-4 inhibits TGF-β-induced Foxp3+ T cells and, together with TGF-β, generates IL-9+ IL-10+ Foxp3− effector T cells. Nature immunology, 9(12), 1347-1355. https://doi.org/10.1038/ni.1677

Dean, J. W., Peters, L. D., Fuhrman, C. A., Seay, H. R., Posgai, A. L., Stimpson, S. E., ... & Brusko, T. M. (2020). Innate inflammation drives NK cell activation to impair Treg activity. Journal of autoimmunity, 108, 102417.

Del Chierico, F., Rapini, N., Deodati, A., Matteoli, M. C., Cianfarani, S., & Putignani, L. (2022). Pathophysiology of type 1 diabetes and gut microbiota role. International Journal of Molecular Sciences, 23(23), 14650. https://doi.org/10.3390/ijms232314650

Diana, J., Simoni, Y., Furio, L., Beaudoin, L., Agerberth, B., Barrat, F., & Lehuen, A. (2013). Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. Nature medicine, 19(1), 65–73. https://doi.org/10.1038/nm.3042

Dong, C. (2011). Genetic controls of Th17 cell differentiation and plasticity. Experimental & molecular medicine, 43(1), 1-6.

Durant, L., Watford, W. T., Ramos, H. L., Laurence, A., Vahedi, G., Wei, L., ... & O'Shea, J. J. (2010). Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity, 32(5), 605-615. DOI: 10.1016/j.immuni.2010.05.003

Eberl, G., Colonna, M., Di Santo, J. P., & McKenzie, A. N. (2015). Innate lymphoid cells: a new paradigm in immunology. Science, 348(6237), aaa6566.

Edner, N. M., Heuts, F., Thomas, N., Wang, C. J., Petersone, L., Kenefeck, R., ... & Walker, L. S. (2020). Follicular helper T cell profiles predict response to costimulation blockade in type 1 diabetes. Nature immunology, 21(10), 1244-1255.

Eisenbarth, G. S. (1986). Type I diabetes mellitus. New England journal of medicine, 314(21), 1360-1368. DOI: 10.1056/NEJM198605223142106

Elliott, J. M., & Yokoyama, W. M. (2011). Unifying concepts of MHC-dependent natural killer cell education. Trends in immunology, 32(8), 364-372. https://doi.org/10.1016/j.it.2011.06.001

Fang, S., Huang, Y., Wang, N., Zhang, S., Zhong, S., Li, Y., ... & Fan, X. (2019). Insights into local orbital immunity: evidence for the involvement of the Th17 cell pathway in thyroid-associated ophthalmopathy. The Journal of Clinical Endocrinology & Metabolism, 104(5), 1697-1711. https://doi.org/10.1210/jc.2018-01626

Ferraro, A., Socci, C., Stabilini, A., Valle, A., Monti, P., Piemonti, L., ... & Battaglia, M. (2011). Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. Diabetes, 60(11), 2903-2913.

Ferreira, R. C., Guo, H., Coulson, R. M., Smyth, D. J., Pekalski, M. L., Burren, O. S., ... & Ziegler, A. G. (2014). A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. Diabetes, 63(7), 2538-2550. https://doi.org/10.2337/db13-1777

Ferreira, R. C., Simons, H. Z., Thompson, W. S., Cutler, A. J., Dopico, X. C., Smyth, D. J., ... & Pekalski, M. L. (2015). IL-21 production by CD4+ effector T cells and frequency of circulating follicular helper T cells are increased in type 1 diabetes patients. Diabetologia, 58(4), 781-790.

Fishilevich, S., Nudel, R., Rappaport, N., Hadar, R., Plaschkes, I., Iny Stein, T., ... & Cohen, D. (2017). GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. Database, 2017, bax028. 10.1093/database/bax028

Fontenot, J., Gavin, M. & Rudensky, A. Foxp3 programs the development and function of CD4+CD25+regulatory T cells. Nat Immunol 4, 330–336 (2003). https://doi.org/10.1038/ni904

Gaffen, S. L., Jain, R., Garg, A. V., & Cua, D. J. (2014). The IL-23–IL-17 immune axis: from mechanisms to therapeutic testing. Nature reviews immunology, 14(9), 585-600. https://doi.org/10.1038/nri3707

Gao, L., Altae-Tran, H., Böhning, F., Makarova, K. S., Segel, M., Schmid-Burgk, J. L., ... & Zhang, F. (2020). Diverse enzymatic activities mediate antiviral immunity in prokaryotes. Science, 369(6507), 1077-1084. DOI: 10.1126/science.aba0372

Gasteiger, G., & Rudensky, A. Y. (2014). Interactions between innate and adaptive lymphocytes. Nature Reviews Immunology, 14(9), 631-639.

Gaublomme, J. T., Yosef, N., Lee, Y., Gertner, R. S., Yang, L. V., Wu, C., ... & Regev, A. (2015). Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. Cell, 163(6), 1400-1412. https://doi.org/10.1016/j.cell.2015.11.009

Ghoreschi, K., Laurence, A., Yang, X. P., Tato, C. M., McGeachy, M. J., Konkel, J. E., ... & O'Shea, J. J. (2010). Generation of pathogenic TH17 cells in the absence of TGF-β signalling. Nature, 467(7318), 967-971.

Ghoreschi, K., Laurence, A., Yang, X. P., Tato, C. M., McGeachy, M. J., Konkel, J. E., ... & O'Shea, J. J. (2010). Generation of pathogenic TH17 cells in the absence of TGF-β signalling. Nature, 467(7318), 967-971. https://doi.org/10.1038/nature09447

Gregory, G. A., Robinson, T. I., Linklater, S. E., Wang, F., Colagiuri, S., de Beaufort, C., ... & Ogle, G. D. (2022). Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. The lancet Diabetes & endocrinology, 10(10), 741-760.

Guan, F., Wang, R., Yi, Z., Luo, P., Liu, W., Xie, Y., ... & Cheng, Q. (2025). Tissue macrophages: origin, heterogenity, biological functions, diseases and therapeutic targets. Signal Transduction and Targeted Therapy, 10(1), 93. https://doi.org/10.1038/s41392-025-02124-y

Hall, J. A., Pokrovskii, M., Kroehling, L., Kim, B. R., Kim, S. Y., Wu, L., ... & Littman, D. R. (2022). Transcription factor RORα enforces stability of the Th17 cell effector program by binding to a Rorc cis-regulatory element. Immunity, 55(11), 2027-2043. https://doi.org/10.1016/j.immuni.2022.09.013

Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W. M., Zheng, S., Butler, A., ... & Satija, R. (2021). Integrated analysis of multimodal single-cell data. Cell, 184(13), 3573-3587. https://doi.org/10.1016/j.cell.2021.04.048

Happel, K. I., Dubin, P. J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L. J., ... & Kolls, J. K. (2005). Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. The Journal of experimental medicine, 202(6), 761-769. https://doi.org/10.1084/jem.20050193

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M., & 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. Nature immunology, 6(11), 1123-1132. https://doi.org/10.1038/ni1254

Health, T. L. D. (2024). Machine learning to predict type 1 diabetes in children. The Lancet. Digital health, 6(6), e374.

Herold, K. C., Bundy, B. N., Long, S. A., Bluestone, J. A., DiMeglio, L. A., Dufort, M. J., Gitelman, S. E., Gottlieb, P. A., Krischer, J. P., Linsley, P. S., Marks, J. B., Moore, W., Moran, A., Rodriguez, H., Russell, W. E., Schatz, D., Skyler, J. S., Tsalikian, E., Wherrett, D. K., Ziegler, A. G., … Type 1 Diabetes TrialNet Study Group (2019). An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. The New England journal of medicine, 381(7), 603–613. https://doi.org/10.1056/NEJMoa1902226

Herold, K. C., Delong, T., Perdigoto, A. L., Biru, N., Brusko, T. M., & Walker, L. S. (2024). The immunology of type 1 diabetes. Nature Reviews Immunology, 24(6), 435-451. https://doi.org/10.1038/s41577-023-00985-4

Hnisz, D., Abraham, B. J., Lee, T. I., Lau, A., Saint-André, V., Sigova, A. A., ... & Young, R. A. (2013). Super-enhancers in the control of cell identity and disease. Cell, 155(4), 934-947. https://doi.org/10.1016/j.cell.2013.09.053

Holland, S. M., DeLeo, F. R., Elloumi, H. Z., Hsu, A. P., Uzel, G., Brodsky, N., ... & Grimbacher, B. (2007). STAT3 mutations in the hyper-IgE syndrome. New England Journal of Medicine, 357(16), 1608-1619. https://doi.org/10.1056/NEJMoa073687

Honardoost, M. A., Adinatha, A., Schmidt, F., Ranjan, B., Ghaeidamini, M., Arul Rayan, N., ... & Prabhakar, S. (2024). Systematic immune cell dysregulation and molecular subtypes revealed by single-cell RNA-seq of subjects with type 1 diabetes. Genome medicine, 16(1), 45. https://doi.org/10.1186/s13073-024-01300-z

Honda, K., & Littman, D. R. (2016). The microbiota in adaptive immune homeostasis and disease. Nature, 535(7610), 75-84.

Honkanen, J., Nieminen, J. K., Gao, R., Luopajarvi, K., Salo, H. M., Ilonen, J., ... & Vaarala, O. (2010). IL-17 immunity in human type 1 diabetes. The Journal of Immunology, 185(3), 1959-1967.

Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science, 299(5609), 1057-1061. https://doi.org/10.1126/science.1079490

Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science, 299(5609), 1057-1061. https://doi.org/10.1126/science.1079490

Hu, D., Notarbartolo, S., Croonenborghs, T., Patel, B., Cialic, R., Yang, T. H., ... & Weiner, H. L. (2017). Transcriptional signature of human pro-inflammatory TH17 cells identifies reduced IL10 gene expression in multiple sclerosis. Nature communications, 8(1), 1600. https://doi.org/10.1038/s41467-017-01571-8

Huang, J., Xiao, Y., Zheng, P., Zhou, W., Wang, Y., Huang, G., ... & Zhou, Z. (2019). Distinct neutrophil counts and functions in newly diagnosed type 1 diabetes, latent autoimmune diabetes in adults, and type 2 diabetes. Diabetes/Metabolism Research and Reviews, 35(1), e3064. https://doi.org/10.1155/2020/4106518

Hull, C. M., Peakman, M., & Tree, T. I. (2017). Regulatory T cell dysfunction in type 1 diabetes: what's broken and how can we fix it?. Diabetologia, 60(10), 1839-1850. https://doi.org/10.1007/s00125-017-4377-1

Imianowski, C. J., Chen, Q., Workman, C. J., & Vignali, D. A. (2025). Regulatory T cells in the tumour microenvironment. Nature Reviews Cancer, 1-20. https://doi.org/10.1038/s41568-025-00832-9

Infante-Duarte, C., Horton, H. F., Byrne, M. C., & Kamradt, T. (2000). Microbial lipopeptides induce the production of IL-17 in Th cells. The Journal of Immunology, 165(11), 6107-6115. https://doi.org/10.4049/jimmunol.165.11.6107

Insel, R. A., Dunne, J. L., Atkinson, M. A., Chiang, J. L., Dabelea, D., Gottlieb, P. A., ... & Ziegler, A. G. (2015). Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes care*, *38*(10), 1964-1974. https://doi.org/10.2337/dc15-1419

Isailovic, N., Daigo, K., Mantovani, A., & Selmi, C. (2015). Interleukin-17 and innate immunity in infections and chronic inflammation. Journal of autoimmunity, 60, 1-11. https://doi.org/10.1016/j.jaut.2015.04.006

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., ... & Iwakura, Y. (2009). Differential roles of interleukin-17A and-17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity, 30(1), 108-119. 10.1016/j.immuni.2008.11.009

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., ... & Littman, D. R. (2006). The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell, 126(6), 1121-1133. https://doi.org/10.1016/j.cell.2006.07.035

Ivanov, I. I., Zhou, L., & Littman, D. R. (2007, December). Transcriptional regulation of Th17 cell differentiation. In Seminars in immunology (Vol. 19, No. 6, pp. 409-417). Academic Press. https://doi.org/10.1016/j.smim.2007.10.011

Jain, R., Chen, Y., Kanno, Y., Joyce-Shaikh, B., Vahedi, G., Hirahara, K., ... & Cua, D. J. (2016). Interleukin-23-induced transcription factor Blimp-1 promotes pathogenicity of T helper 17 cells. Immunity, 44(1), 131-142. 10.1016/j.immuni.2015.11.009

Jia, X., & Yu, L. (2024). Understanding islet autoantibodies in prediction of type 1 diabetes. Journal of the Endocrine Society, 8(1), bvad160. https://doi.org/10.1210/jendso/bvad160

Jiang, Y., Liu, Y., Lu, H., Sun, S. C., Jin, W., Wang, X., & Dong, C. (2018). Epigenetic activation during T helper 17 cell differentiation is mediated by Tripartite motif containing 28. Nature communications, 9(1), 1-13. https://doi.org/10.1038/s41467-018-03852-2

Kalim, U. U., Biradar, R., Junttila, S., Khan, M. M., Tripathi, S., Khan, M. H., ... & Lahesmaa, R. (2024). A proximal enhancer regulates RORA expression during early human Th17 cell differentiation. Clinical Immunology, 264, 110261. https://doi.org/10.1016/j.clim.2024.110261

Kallionpää, H., Elo, L. L., Laajala, E., Mykkänen, J., Ricano-Ponce, I., Vaarma, M., ... & Lahesmaa, R. (2014). Innate immune activity is detected prior to seroconversion in children with HLA-conferred type 1 diabetes susceptibility. Diabetes, 63(7), 2402-2414. https://doi.org/10.2337/db13-1775

Kallionpää, H., Somani, J., Tuomela, S., Ullah, U., De Albuquerque, R., Lönnberg, T., ... & Lahesmaa, R. (2019). Early detection of peripheral blood cell signature in children developing β-cell autoimmunity at a young age. Diabetes, 68(10), 2024-2034. https://doi.org/10.2337/db19-0287

Kan, A. K. C., Tang, W. T., & Li, P. H. (2024). Helper T cell subsets: Development, function and clinical role in hypersensitivity reactions in the modern perspective. Heliyon, 10(9).

Kanayama, M., Izumi, Y., Onai, N., Akashi, T., Hiraoka, Y., & Ohteki, T. (2025). Diverse developmental pathways of lymphoid conventional dendritic cells with distinct tissue distribution and function. Science Advances, 11(23), eadt4909.

Kaplan, M. H., Hufford, M. M., & Olson, M. R. (2015). The development and in vivo function of T helper 9 cells. Nature Reviews Immunology, 15(5), 295-307.

Katagiri, T., Kameda, H., Nakano, H., & Yamazaki, S. (2021). Regulation of T cell differentiation by the AP-1 transcription factor JunB. Immunological medicine, 44(3), 197-203. https://doi.org/10.1080/25785826.2021.1872838

Katz, J. D., Benoist, C., & Mathis, D. (1995). T helper cell subsets in insulin-dependent diabetes. Science, 268(5214), 1185-1188.

Kenefeck, R., Wang, C. J., Kapadi, T., Wardzinski, L., Attridge, K., Clough, L. E., ... & Walker, L. S. (2015). Follicular helper T cell signature in type 1 diabetes. The Journal of clinical investigation, 125(1), 292-303.

Khader, S. A., Bell, G. K., Pearl, J. E., Fountain, J. J., Rangel-Moreno, J., Cilley, G. E., ... & Cooper, A. M. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nature immunology, 8(4), 369-377.

Khan, M. M., Khan, M. H., Kalim, U. U., Khan, S., Junttila, S., Paulin, N., ... & Lahesmaa, R. (2022). Long intergenic noncoding RNA MIAT as a regulator of human Th17 cell differentiation. Frontiers in Immunology, 13, 856762. https://doi.org/10.3389/fimmu.2022.856762

Khattri, R., Cox, T., Yasayko, S. A., & Ramsdell, F. (2003). An essential role for Scurfin in CD4+ CD25+ T regulatory cells. Nature immunology, 4(4), 337-342. https://doi.org/10.1038/ni909

Kinugasa, T., Sakaguchi, T., Gu, X., & Reinecker, H. C. (2000). Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology, 118(6), 1001-1011. https://doi.org/10.1016/S0016-5085(00)70351-9

Knip, M. (2021). Type 1 diabetes in Finland: past, present, and future. The Lancet Diabetes & Endocrinology, 9(5), 259-260. https://doi.org/10.1016/S2213-8587(21)00074-7

Korn, T., Bettelli, E., Gao, W., Awasthi, A., Jäger, A., Strom, T. B., Oukka, M., & Kuchroo, V. K. (2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature, 448(7152), 484–487. https://doi.org/10.1038/nature05970

Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V. K. (2009). IL-17 and Th17 Cells. Annual review of immunology, 27(1), 485-517.

Krakowski, M., & Owens, T. (1996). Interferon-γ confers resistance to experimental allergic encephalomyelitis. European journal of immunology, 26(7), 1641-1646. https://doi.org/10.1002/eji.1830260735

Lam, N., Lee, Y., & Farber, D. L. (2024). A guide to adaptive immune memory. Nature Reviews Immunology, 24(11), 810-829. https://doi.org/10.1038/s41577-024-01040-6

Landry, L. G., Anderson, A. M., Russ, H. A., Yu, L., Kent, S. C., Atkinson, M. A., ... & Nakayama, M. (2021). Proinsulin-reactive CD4 T cells in the islets of type 1 diabetes organ donors. Frontiers in endocrinology, 12, 622647. doi: 10.3389/fendo.2021.622647

Langrish, C. L., Chen, Y., Blumenschein, W. M., Mattson, J., Basham, B., Sedgwick, J. D., ... & Cua, D. J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. The Journal of experimental medicine, 201(2), 233-240. https://doi.org/10.1084/jem.20041257

Lee, H. J., Takemoto, N., Kurata, H., Kamogawa, Y., Miyatake, S., O'Garra, A., & Arai, N. (2000). GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. The Journal of experimental medicine, 192(1), 105-116. https://doi.org/10.1084/jem.192.1.105

Lee, W., & Lee, G. R. (2018). Transcriptional regulation and development of regulatory T cells. Experimental & molecular medicine, 50(3), e456-e456. https://doi.org/10.1038/emm.2017.313

Leete, P., Oram, R. A., McDonald, T. J., Shields, B. M., Ziller, C., TIGI study team Roep Bart O. Tree Timothy I. Patel Kashyap Hammersley Suzy Bolt Robert Hill Anita V., ... & Morgan, N. G. (2020). Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia, 63(6), 1258-1267. https://doi.org/10.1007/s00125-020-05115-6

Lernmark, Å., Agardh, D., Akolkar, B., Gesualdo, P., Hagopian, W. A., Haller, M. J., ... & Krischer, J. (2025). Looking back at the TEDDY study: lessons and future directions. Nature Reviews Endocrinology, 21(3), 154-165. 10.1038/s41574-024-01045-0

Li, P., Spolski, R., Liao, W., Wang, L., Murphy, T. L., Murphy, K. M., & Leonard, W. J. (2012). BATF–JUN is critical for IRF4-mediated transcription in T cells. Nature, 490(7421), 543-546. https://doi.org/10.1038/nature11530

Limagne, E., Thibaudin, M., Euvrard, R., Berger, H., Chalons, P., Vegan, F., ... & Ghiringhelli, F. (2017). Sirtuin-1 activation controls tumor growth by impeding Th17 differentiation via STAT3 deacetylation. Cell reports, 19(4), 746-759. https://doi.org/10.1016/j.celrep.2017.04.004

Lin, F., Meng, X., Guo, Y., Cao, W., Liu, W., Xia, Q., ... & Wang, L. (2019). Epigenetic initiation of the TH17 differentiation program is promoted by Cxxc finger protein 1. Science Advances, 5(10), eaax1608. DOI: 10.1126/sciadv.aax1608

Lin, J., Moradi, E., Salenius, K., Lehtipuro, S., Häkkinen, T., Laiho, J. E., ... & TEDDY Study Group. (2023). Distinct transcriptomic profiles in children prior to the appearance of type 1 diabetes-linked islet autoantibodies and following enterovirus infection. Nature Communications, 14(1), 7630. https://doi.org/10.1038/s41467-023-42763-9

Liu, C., Workman, C. J., & Vignali, D. A. (2016). Targeting regulatory T cells in tumors. The FEBS journal, 283(14), 2731-2748. https://doi.org/10.1111/febs.13656

Lohoff, M., Mittrücker, H. W., Prechtl, S., Bischof, S., Sommer, F., Kock, S., ... & Mak, T. W. (2002). Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4. Proceedings of the National Academy of Sciences, 99(18), 11808-11812. DOI: 10.1073/pnas.182425099

Marshall, J. S., Warrington, R., Watson, W., & Kim, H. L. (2018). An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology, 14(Suppl 2), 49. https://doi.org/10.1186/s13223-018-0278-1

Martinov, T., & Fife, B. T. (2020). Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance. Annals of the New York Academy of Sciences, 1461(1), 73-103. https://doi.org/10.1111/nyas.14106

Marwaha, A. K., Crome, S. Q., Panagiotopoulos, C., Berg, K. B., Qin, H., Ouyang, Q., ... & Tan, R. (2010). Cutting edge: increased Il-17–secreting T cells in children with new-onset type 1 diabetes. The Journal of Immunology, 185(7), 3814-3818.

Mathieu, C., Gillard, P., & Benhalima, K. (2017). Insulin analogues in type 1 diabetes mellitus: getting better all the time. Nature Reviews Endocrinology, 13(7), 385-399. https://doi.org/10.1038/nrendo.2017.39

Matthias, J., & Zielinski, C. E. (2019). Shaping the diversity of Th2 cell responses in epithelial tissues and its potential for allergy treatment. European journal of immunology, 49(9), 1321-1333. https://doi.org/10.1002/eji.201848011

McClymont, S. A., Putnam, A. L., Lee, M. R., Esensten, J. H., Liu, W., Hulme, M. A., ... & Brusko, T. M. (2011). Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. The Journal of Immunology, 186(7), 3918-3926.

McGeachy, M. J., Bak-Jensen, K. S., Chen, Y. I., Tato, C. M., Blumenschein, W., McClanahan, T., & Cua, D. J. (2007). TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell–mediated pathology. Nature immunology, 8(12), 1390-1397. https://doi.org/10.1038/ni1539

Michels, A. W., Brusko, T. M., Evans-Molina, C., Homann, D., Richardson, S. J., & Powers, A. C. (2025). Challenges and Opportunities for understanding the pathogenesis of type 1 diabetes: an Endocrine Society Scientific Statement. The Journal of Clinical Endocrinology & Metabolism, 110(9), 2496-2508. https://doi.org/10.1210/clinem/dgaf267

Mikami, N., Kawakami, R., Chen, K. Y., Sugimoto, A., Ohkura, N., & Sakaguchi, S. (a2020). Epigenetic conversion of conventional T cells into regulatory T cells by CD28 signal deprivation. Proceedings of the National Academy of Sciences, 117(22), 12258-12268. https://doi.org/10.1073/pnas.1922600117

Mikami, N., Kawakami, R., Sugimoto, A., Arai, M., & Sakaguchi, S. (b2025). Generating functionally stable and antigen-specific Treg cells from effector T cells for cell therapy of inflammatory diseases. Science Translational Medicine, 17(821), eadr6049. DOI: 10.1126/scitranslmed.adr6049

Mills, K. H. (2023). IL-17 and IL-17-producing cells in protection versus pathology. Nature Reviews Immunology, 23(1), 38-54.

Milner, J. D., Brenchley, J. M., Laurence, A., Freeman, A. F., Hill, B. J., Elias, K. M., ... & Douek, D. C. (2008). Impaired TH17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature, 452(7188), 773-776. https://doi.org/10.1038/nature06764

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., ... & Karasuyama, H. (2007). Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature, 448(7157), 1058-1062. https://doi.org/10.1038/nature06096

Monin, L., & Gaffen, S. L. (2018). Interleukin 17 family cytokines: signaling mechanisms, biological activities, and therapeutic implications. Cold Spring Harbor perspectives in biology, 10(4), a028522.

Moon, Y. M., Lee, S. Y., Kwok, S. K., Lee, S. H., Kim, D., Kim, W. K., ... & Cho, M. L. (2017). The Fos-related antigen 1–JUNB/activator protein 1 transcription complex, a downstream target of signal transducer and activator of transcription 3, induces T helper 17 differentiation and promotes experimental autoimmune arthritis. Frontiers in immunology, 8, 1793. https://doi.org/10.3389/fimmu.2017.01793

Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., & Coffman, R. L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. Journal of Immunology (Baltimore, MD.: 1950), 136(7), 2348-2357. https://doi.org/10.4049/jimmunol.136.7.2348

Mukasa, R., Balasubramani, A., Lee, Y. K., Whitley, S. K., Weaver, B. T., Shibata, Y., Crawford, G. E., Hatton, R. D., & Weaver, C. T. (2010). Epigenetic instability of cytokine and transcription factor gene loci underlies plasticity of the T helper 17 cell lineage. Immunity, 32(5), 616–627. https://doi.org/10.1016/j.immuni.2010.04.016

Muranski, P., & Restifo, N. P. (2013). Essentials of Th17 cell commitment and plasticity. Blood, The Journal of the American Society of Hematology, 121(13), 2402-2414. https://doi.org/10.1182/blood-2012-09-378653

Murphy, C. A., Langrish, C. L., Chen, Y., Blumenschein, W., McClanahan, T., Kastelein, R. A., ... & Cua, D. J. (2003). Divergent pro-and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. The Journal of experimental medicine, 198(12), 1951-1957. https://doi.org/10.1084/jem.20030896

Nakayasu, E. S., Qian, W. J., Evans-Molina, C., Mirmira, R. G., Eizirik, D. L., & Metz, T. O. (2019). The role of proteomics in assessing beta-cell dysfunction and death in type 1 diabetes. Expert Review of Proteomics, 16(7), 569–582. https://doi.org/10.1080/14789450.2019.1634548

Nekoua, M. P., Alidjinou, E. K., & Hober, D. (2022). Persistent coxsackievirus B infection and pathogenesis of type 1 diabetes mellitus. Nature Reviews Endocrinology, 18(8), 503-516. https://doi.org/10.1038/s41574-022-00688-1

Niederlova, V., Neuwirth, A., Neuman, V., Michalik, J., Charvatova, B., Modrak, M., ... & Stepanek, O. (2025). Imbalance of stem-like and effector T cell states in children with early type 1 diabetes across conventional and regulatory subsets. Nat Commun 16, 11301 (2025). https://doi.org/10.1038/s41467-025-66459-4

Noble, J. A., & Valdes, A. M. (2011). Genetics of the HLA region in the prediction of type 1 diabetes. Current diabetes reports, 11(6), 533-542.

Notti, R. Q., Yi, F., Heissel, S., Bush, M. W., Molvi, Z., Das, P., ... & Walz, T. (2025). The resting and ligand-bound states of the membrane-embedded human T-cell receptor–CD3 complex. Nature Communications, 16(1), 10996. https://doi.org/10.1038/s41467-025-66939-7

Ogawa, A., Andoh, A., Araki, Y., Bamba, T., & Fujiyama, Y. (2004). Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice. Clinical immunology, 110(1), 55-62. https://doi.org/10.1016/j.clim.2003.09.013

Ogawa, C., Bankoti, R., Nguyen, T., Hassanzadeh-Kiabi, N., Nadeau, S., Porritt, R. A., ... & Martins, G. A. (2018). Blimp-1 functions as a molecular switch to prevent inflammatory activity in Foxp3+ RORγt+ regulatory T cells. *Cell reports*, *25*(1), 19-28. DOI: 10.1016/j.celrep.2018.09.016 External Link

Okamura, T., Kitagawa, N., Kitagawa, N., Sakai, K., Sumi, M., Kobayashi, G., ... & Fukui, M. (2024). Single-cell analysis reveals islet autoantigen's immune activation in type 1 diabetes patients. Journal of Clinical Biochemistry and Nutrition, 76(1), 64. https://doi.org/10.3164/jcbn.24-86

Oppmann, B., Lesley, R., Blom, B., Timans, J. C., Xu, Y., Hunte, B., ... & Kastelein, R. A. (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(5), 715-725. DOI: 10.1016/s1074-7613(00)00070-4

Oshima, M., Halliez, C., Kobaisi, F., Modé, N., Fouque, A., Brandao, B., ... & Scharfmann, R. (2026). Killing of Human β-Cells by CD8+ T Cells Triggers Inflammatory Paracrine Signaling and Neighboring β-Cell Dysfunction. Diabetes, 75(1), 124-132. https://doi.org/10.2337/db25-0473

Owen, J. A, J, Punt, S. A, Stranford, and P. P Jones. 2018. Kuby Immunology.

Paradowska-Gorycka, A., Wajda, A., Romanowska-Próchnicka, K., Walczuk, E., Kuca-Warnawin, E., Kmiolek, T., ... & Pawlik, A. (2020). Th17/Treg-related transcriptional factor expression and cytokine profile in patients with rheumatoid arthritis. *Frontiers in immunology*, *11*, 572858. doi: 10.3389/fimmu.2020.572858

Park, E., & Ciofani, M. (2025). Th17 cell pathogenicity in autoimmune disease. Experimental & Molecular Medicine, 57(9), 1913-1927. https://doi.org/10.1038/s12276-025-01535-9

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y. H., ... & Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nature immunology, 6(11), 1133-1141. https://doi.org/10.1038/ni1261

Park, S. A., Lim, Y. J., Ku, W. L., Zhang, D., Cui, K., Tang, L. Y., ... & Chen, W. (2022). Opposing functions of circadian protein DBP and atypical E2F family E2F8 in anti-tumor Th9 cell differentiation. Nature Communications, 13(1), 6069. https://doi.org/10.1038/s41467-022-33733-8

Pathiraja, V., Kuehlich, J. P., Campbell, P. D., Krishnamurthy, B., Loudovaris, T., Coates, P. T. H., ... & Mannering, S. I. (2015). Proinsulin-specific, HLA-DQ8, and HLA-DQ8-transdimer–restricted CD4+ T cells infiltrate islets in type 1 diabetes. Diabetes, 64(1), 172-182.

Perdiguero, E. G., Klapproth, K., Schulz, C., Busch, K., de Bruijn, M., Rodewald, H. R., & Geissmann, F. (2015). The origin of tissue-resident macrophages: when an erythro-myeloid progenitor is an erythro-myeloid progenitor. *Immunity*, *43*(6), 1023-1024. DOI: 10.1016/j.immuni.2015.11.022

Peters, A., Lee, Y., & Kuchroo, V. K. (2011). The many faces of Th17 cells. Current opinion in immunology, 23(6), 702-706.

Petrich de Marquesini, L. G., Fu, J., Connor, K. J., Bishop, A. J., McLintock, N. E., Pope, C., ... & Dayan, C. M. (2010). IFN-γ and IL-10 islet-antigen-specific T cell responses in autoantibody-negative first-degree relatives of patients with type 1 diabetes. Diabetologia, 53(7), 1451-1460.

Poirot, L., Benoist, C., & Mathis, D. (2004). Natural killer cells distinguish innocuous and destructive forms of pancreatic islet autoimmunity. Proceedings of the National Academy of Sciences, 101(21), 8102-8107. https://doi.org/10.1073/pnas.0402065101

Renaude, E., Kroemer, M., Loyon, R., Binda, D., Borg, C., Guittaut, M., ... & Peixoto, P. (2020). The fate of Th17 cells is shaped by epigenetic modifications and remodeled by the tumor microenvironment. International journal of molecular sciences, 21(5), 1673. 10.3390/ijms21051673

Robertson, C. C., Inshaw, J. R., Onengut-Gumuscu, S., Chen, W. M., Santa Cruz, D. F., Yang, H., ... & Rich, S. S. (2021). Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. Nature genetics, 53(7), 962-971. https://doi.org/10.1038/s41588-021-00880-5

Roep, B. O., Thomaidou, S., Van Tienhoven, R., & Zaldumbide, A. (2021). Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nature Reviews Endocrinology, 17(3), 150-161. https://doi.org/10.1038/s41574-020-00443-4

Rogozynski, N. P., & Dixon, B. (2024). The Th1/Th2 paradigm: A misrepresentation of helper T cell plasticity. Immunology letters, 268, 106870. https://doi.org/10.1016/j.imlet.2024.106870

Rojas, J., Bermudez, V., Palmar, J., Martínez, M. S., Olivar, L. C., Nava, M., ... & Velasco, M. (2018). Pancreatic beta cell death: novel potential mechanisms in diabetes therapy. Journal of diabetes research, 2018(1), 9601801.

Rouxel, O., Da Silva, J., Beaudoin, L., Nel, I., Tard, C., Cagninacci, L., ... & Lehuen, A. (2017). Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. Nature immunology, 18(12), 1321-1331. https://doi.org/10.1038/ni.3854

Rudner, X. L., Happel, K. I., Young, E. A., & Shellito, J. E. (2007). Interleukin-23 (IL-23)-IL-17 cytokine axis in murine Pneumocystis carinii infection. Infection and immunity, 75(6), 3055-3061.

Saijo, S., Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., ... & Iwakura, Y. (2010). Dectin-2 recognition of α-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. Immunity, 32(5), 681-691.

Schnell, A., Littman, D. R., & Kuchroo, V. K. (2023). TH17 cell heterogeneity and its role in tissue inflammation. Nature immunology, 24(1), 19-29.

Schraml, B. U., Hildner, K., Ise, W., Lee, W. L., Smith, W. A. E., Solomon, B., ... & Murphy, K. M. (2009). The AP-1 transcription factor Batf controls TH17 differentiation. Nature, 460(7253), 405-409. https://doi.org/10.1038/nature08114

Sen, A., Youssef, S., Wendt, K., & Anakk, S. (2023). Depletion of IQ motif-containing GTPase activating protein 2 (IQGAP2) reduces hepatic glycogen and impairs insulin signaling. Journal of Biological Chemistry, 299(11). doi:10.1016/j.jbc.2023.105322

Shah, K., Al-Haidari, A., Sun, J., & Kazi, J. U. (2021). T cell receptor (TCR) signaling in health and disease. Signal transduction and targeted therapy, 6(1), 412. https://doi.org/10.1038/s41392-021-00823-w

Shetty, A., Bhosale, S. D., Tripathi, S. K., Buchacher, T., Biradar, R., Rasool, O., ... & Lahesmaa, R. (2021). Interactome networks of FOSL1 and FOSL2 in human Th17 cells. ACS omega, 6(38), 24834-24847. https://doi.org/10.1021/acsomega.1c03681

Shetty, A., Tripathi, S. K., Junttila, S., Buchacher, T., Biradar, R., Bhosale, S. D., ... & Lahesmaa, R. (2022). A systematic comparison of FOSL1, FOSL2 and BATF-mediated transcriptional regulation during early human Th17 differentiation. Nucleic acids research, 50(9), 4938-4958. https://doi.org/10.1093/nar/gkac256

Shevach, E. M., & Thornton, A. M. (2014). tTregs, pTregs, and iTregs: similarities and differences. Immunological reviews, 259(1), 88-102.

Skowera, A., Ellis, R. J., Varela-Calviño, R., Arif, S., Huang, G. C., Van-Krinks, C., ... & Peakman, M. (2008). CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. The Journal of clinical investigation, 118(10), 3390-3402. https://doi.org/10.1172/JCI35449

Smith, M. J., Packard, T. A., O'Neill, S. K., Henry Dunand, C. J., Huang, M., Fitzgerald-Miller, L., ... & Cambier, J. C. (2015). Loss of anergic B cells in prediabetic and new-onset type 1 diabetic patients. Diabetes, 64(5), 1703-1712. https://doi.org/10.2337/db13-1798

Song, X., Gao, H., & Qian, Y. (2014). Th17 differentiation and their pro-inflammation function. T helper cell differentiation and their function, 99-151.

Starskaia, I., Laajala, E., Grönroos, T., Härkönen, T., Junttila, S., Kattelus, R., ... & Lahesmaa, R. (2022). Early DNA methylation changes in children developing beta cell autoimmunity at a young age. Diabetologia, 65(5), 844-860. DOI: 10.1007/s00125-022-05657-x

Starskaia, I., Valta, M., Pietilä, S., Suomi, T., Pahkuri, S., Kalim, U. U., ... & Lahesmaa, R. (2024). Distinct cellular immune responses in children en route to type 1 diabetes with different first-appearing autoantibodies. Nature Communications, 15(1), 3810. https://doi.org/10.1038/s41467-024-47918-w

Staudt, V., Bothur, E., Klein, M., Lingnau, K., Reuter, S., Grebe, N., ... & Bopp, T. (2010). Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. Immunity, 33(2), 192-202. doi:10.1016/j.immuni.2010.07.014

Stene, L. C. (2024). Global diabetes incidence trends in young adults. The Lancet Diabetes & Endocrinology, 12(12), 870-872. DOI: 10.1016/S2213-8587(24)00286-9

Stockinger, B., & Omenetti, S. (2017). The dichotomous nature of T helper 17 cells. Nature Reviews Immunology, 17(9), 535-544. https://doi.org/10.1038/nri.2017.50

Sun, L., Su, Y., Jiao, A., Wang, X., & Zhang, B. (2023). T cells in health and disease. Signal transduction and targeted therapy, 8(1), 235.

Sun, L., Xi, S., He, G., Li, Z., Gang, X., Sun, C., ... & Wang, G. (2020). Two to tango: dialogue between adaptive and innate immunity in type 1 diabetes. Journal of diabetes research, 2020(1), 4106518.

Suomi, T., Seyednasrollah, F., Jaakkola, M. K., Faux, T., & Elo, L. L. (2017). ROTS: An R package for reproducibility-optimized statistical testing. PLoS computational biology, 13(5), e1005562. doi: 10.1371/journal.pcbi.1005562

Tatovic, D., Narendran, P., & Dayan, C. M. (2023). A perspective on treating type 1 diabetes mellitus before insulin is needed. Nature Reviews Endocrinology, 19(6), 361-370.

Thakore, P. I., Schnell, A., Huang, L., Zhao, M., Hou, Y., Christian, E., ... & Regev, A. (2024). BACH2 regulates diversification of regulatory and proinflammatory chromatin states in TH17 cells. Nature Immunology, 25(8), 1395-1410. https://doi.org/10.1038/s41590-024-01901-1

Thieu, V. T., Yu, Q., Chang, H. C., Yeh, N., Nguyen, E. T., Sehra, S., & Kaplan, M. H. (2008). Stat4 is required for T-bet to promote IL-12-dependent Th1 fate determination. Immunity, 29(5), 679. DOI:10.1016/j.immuni.2008.08.017

Tian, Y., Han, C., Wei, Z., Dong, H., Shen, X., Cui, Y., ... & Wu, Y. (2021). SOX-5 activates a novel RORγt enhancer to facilitate experimental autoimmune encephalomyelitis by promoting Th17 cell differentiation. Nature Communications, 12(1), 481. https://doi.org/10.1038/s41467-020-20786-w

Togashi, Y., Shitara, K. & Nishikawa, H. Regulatory T cells in cancer immunosuppression — implications for anticancer therapy. Nat Rev Clin Oncol 16, 356–371 (2019). https://doi.org/10.1038/s41571-019-0175-7

Tran, E. H., Prince, E. N., & Owens, T. (2000). IFN-γ shapes immune invasion of the central nervous system via regulation of chemokines. The Journal of Immunology, 164(5), 2759-2768. https://doi.org/10.4049/jimmunol.164.5.2759

TRIGR Study Group. (2007). Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR). Pediatric diabetes, 8(3), 117-137. 10.1111/j.1399-5448.2007.00239.x

Tripathi, S. K., Chen, Z., Larjo, A., Kanduri, K., Nousiainen, K., Äijo, T., ... & Lahesmaa, R. (2017). Genome-wide analysis of STAT3-mediated transcription during early human Th17 cell differentiation. Cell reports, 19(9), 1888-1901. DOI: 10.1016/j.celrep.2017.05.013

Trujillo-Ochoa, J. L., Kazemian, M., & Afzali, B. (2023). The role of transcription factors in shaping regulatory T cell identity. Nature Reviews Immunology, 23(12), 842-856.

Tuomela, S., Rautio, S., Ahlfors, H., Öling, V., Salo, V., Ullah, U., ... & Lahesmaa, R. (2016). Comparative analysis of human and mouse transcriptomes of Th17 cell priming. Oncotarget, 7(12), 13416. https://doi.org/10.18632/oncotarget.7963

Turley, S., Poirot, L., Hattori, M., Benoist, C., & Mathis, D. (2003). Physiological β cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. The Journal of experimental medicine, 198(10), 1527-1537. https://doi.org/10.1084/jem.20030966

Turtinen, M., Härkönen, T., Parkkola, A., Ilonen, J., Knip, M., & Finnish Pediatric Diabetes Register. (2019). Characteristics of familial type 1 diabetes: effects of the relationship to the affected family member on phenotype and genotype at diagnosis. Diabetologia, 62(11), 2025-2039. https://doi.org/10.1007/s00125-019-4952-8

Vahedi, G., Kanno, Y., Furumoto, Y., Jiang, K., Parker, S. C., Erdos, M. R., ... & O'Shea, J. J. (2015). Super-enhancers delineate disease-associated regulatory nodes in T cells. Nature, 520(7548), 558-562. https://doi.org/10.1038/nature14154

Van de Sande, B., Flerin, C., Davie, K., De Waegeneer, M., Hulselmans, G., Aibar, S., ... & Aerts, S. (2020). A scalable SCENIC workflow for single-cell gene regulatory network analysis. Nature protocols, 15(7), 2247-2276. https://doi.org/10.1038/s41596-020-0336-2

Vecchio, F., Buono, N. L., Stabilini, A., Nigi, L., Dufort, M. J., Geyer, S., ... & Type 1 Diabetes TrialNet Study Group. (2018). Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes. JCI insight, 3(18), e122146. https://doi.org/10.1172/jci.insight.122146

Vecchione, A., Jofra, T., Gerosa, J., Shankwitz, K., Di Fonte, R., Galvani, G., ... & Fousteri, G. (2021). Reduced follicular regulatory T cells in spleen and pancreatic lymph nodes of patients with type 1 diabetes. Diabetes, 70(12), 2892-2902.

Veldhoen, M., Uyttenhove, C., Van Snick, J., Helmby, H., Westendorf, A., Buer, J., ... & Stockinger, B. (2008). Transforming growth factor-β'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9–producing subset. Nature immunology, 9(12), 1341-1346.

Viisanen, T., Ihantola, E. L., Näntö-Salonen, K., Hyöty, H., Nurminen, N., Selvenius, J., ... & Kinnunen, T. (2017). Circulating CXCR5+ PD-1+ ICOS+ follicular T helper cells are increased close to the diagnosis of type 1 diabetes in children with multiple autoantibodies. Diabetes, 66(2), 437-447.

Walker, L. S., & von Herrath, M. (2016). CD4 T cell differentiation in type 1 diabetes. Clinical & Experimental Immunology, 183(1), 16-29. https://doi.org/10.1111/cei.12672

Wang, J., Zhao, X., & Wan, Y. Y. (2023). Intricacies of TGF-β signaling in Treg and Th17 cell biology. Cellular & molecular immunology, 20(9), 1002-1022.

Wang, L., Liang, Y., Zhao, C., Ma, P., Zeng, S., Ju, D., ... & Shi, Y. (2025). Regulatory T cells in homeostasis and disease: molecular mechanisms and therapeutic potential. Signal Transduction and Targeted Therapy, 10(1), 345. https://doi.org/10.1038/s41392-025-02326-4

Wang, R., Campbell, S., Amir, M., Mosure, S. A., Bassette, M. A., Eliason, A., ... & Solt, L. A. (2021). Genetic and pharmacological inhibition of the nuclear receptor RORα regulates TH17 driven inflammatory disorders. Nature communications, 12(1), 76. https://doi.org/10.1038/s41467-020-20385-9

Wang, R., Lan, C., Benlagha, K., Camara, N. O. S., Miller, H., Kubo, M., ... & Liu, C. (2024). The interaction of innate immune and adaptive immune system. MedComm, 5(10), e714.

Weaver, C. T., Elson, C. O., Fouser, L. A., & Kolls, J. K. (2013). The Th17 pathway and inflammatory diseases of the intestines, lungs, and skin. Annual Review of Pathology: Mechanisms of Disease, 8(1), 477-512.

Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., ... & Zhao, K. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity, 30(1), 155-167. https://doi.org/10.1016/j.immuni.2008.12.009

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., ... & de Waal Malefyt, R. (2007). Development, cytokine profile and function of human interleukin 17–producing helper T cells. Nature immunology, 8(9), 950-957. https://doi.org/10.1038/ni1497

Wing, J. B., Tanaka, A., & Sakaguchi, S. (2019). Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer. Immunity, 50(2), 302-316.

Wong, F. S., Wen, L. I., Tang, M., Ramanathan, M., Visintin, I., Daugherty, J., ... & Shlomchik, M. J. (2004). Investigation of the role of B-cells in type 1 diabetes in the NOD mouse. Diabetes, 53(10), 2581-2587.

Wu, X., Tian, J., & Wang, S. (2018). Insight into non-pathogenic Th17 cells in autoimmune diseases. Frontiers in immunology, 9, 1112.

Yang, J., Wen, X., Xu, H., Torres-Chinn, N., Speake, C., Greenbaum, C. J., ... & Kwok, W. W. (2017). Antigen-specific T cell analysis reveals that active immune responses to β cell antigens are focused on a unique set of epitopes. The Journal of Immunology, 199(1), 91-96.

Yang, L., Anderson, D. E., Baecher-Allan, C., Hastings, W. D., Bettelli, E., Oukka, M., ... & Hafler, D. A. (2008). IL-21 and TGF-β are required for differentiation of human TH17 cells. Nature, 454(7202), 350-352. https://doi.org/10.1038/nature07021

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., ... & Dong, C. (2008). T helper 17 lineage differentiation is programmed by orphan nuclear receptors RORα and RORγ. Immunity, 28(1), 29-39.

Yosef, N., Shalek, A. K., Gaublomme, J. T., Jin, H., Lee, Y., Awasthi, A., ... & Regev, A. (2013). Dynamic regulatory network controlling TH17 cell differentiation. Nature, 496(7446), 461-468.

Yu, D., Rao, S., Tsai, L. M., Lee, S. K., He, Y., Sutcliffe, E. L., ... & Vinuesa, C. G. (2009). The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity, 31(3), 457-468. https://doi.org/10.1016/j.immuni.2009.07.002

Zhang, F., Meng, G., & Strober, W. (2008). Interactions among the transcription factors Runx1, RORγt and Foxp3 regulate the differentiation of interleukin 17–producing T cells. Nature immunology, 9(11), 1297-1306. https://doi.org/10.1038/ni.1663

Zhang, F., Xia, Y., Su, J., Quan, F., Zhou, H., Li, Q., ... & Jiang, Z. (2024). Neutrophil diversity and function in health and disease. Signal transduction and targeted therapy, 9(1), 343. https://doi.org/10.1038/s41392-024-02049-y

Zhang, W., Liu, X., Zhu, Y., Liu, X., Gu, Y., Dai, X., & Li, B. (2021). Transcriptional and posttranslational regulation of Th17/Treg balance in health and disease. European journal of immunology, 51(9), 2137-2150.

Zhang, X., Olsen, N., & Zheng, S. G. (2020). The progress and prospect of regulatory T cells in autoimmune diseases. Journal of Autoimmunity, 111, 102461.

Zhong, T., Li, X., Lei, K., Tang, R., Deng, Q., Love, P. E., ... & Li, X. (2024). TGF-β-mediated crosstalk between TIGIT+ Tregs and CD226+ CD8+ T cells in the progression and remission of type 1 diabetes. Nature Communications, 15(1), 8894. https://doi.org/10.1038/s41467-024-53264-8

Zhou, L., & Littman, D. R. (2009). Transcriptional regulatory networks in Th17 cell differentiation. Current opinion in immunology, 21(2), 146-152.

Zhu, J. (2018). T helper cell differentiation, heterogeneity, and plasticity. Cold Spring Harbor perspectives in biology, 10(10), a030338.

Zielinski, C. E. (2023). T helper cell subsets: diversification of the field. European journal of immunology, 53(12), 2250218.

Zielinski, C. E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., ... & Sallusto, F. (2012). Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. Nature, 484(7395), 514-518.

Zou, D., Li, X. C., & Chen, W. (2025). Beyond T-cell subsets: stemness and adaptation redefining immunity and immunotherapy. Cellular & Molecular Immunology, 22(9), 957-974.

# List of Figures

## Figures

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2026

ISBN 978-952-02-0668-0 (PRINT)
ISBN 978-952-02-0669-7 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)