DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
304

EPP KALEVISTE

Genetic variants revealing the role of STAT1/STAT3 signaling cytokines in immune protection and pathology

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
304

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
304

EPP KALEVISTE

Genetic variants revealing the role of STAT1/STAT3 signaling cytokines in immune protection and pathology

UNIVERSITY OF TARTU
Press

Molecular Pathology Research Group, Institute of Biomedicine and Translational Medicine, University of Tartu.

The dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Medicine on June 17th, 2020 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

Supervisors: Kai Kisand, MD, PhD, Research Professor of Cellular Immunology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia

Pärt Peterson, PhD, Head of Department of Biomedicine, Professor of Molecular Immunology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia

Lili Milani, PhD, Research Professor of Epi- and Pharmacogenomics, Institute of Genomics, Estonian Genome Center, University of Tartu, Estonia

Reviewers: Reet Mändar, PhD, Professor of Medical Microbial Ecology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia

Katrin Õunap, MD, PhD, Professor of Clinical Genetics, Institute of Clinical Medicine, Faculty of Medicine, University of Tartu, Estonia

Opponent: Tadej Avcin, MD, PhD, Professor of Pediatrics, Department of Allergology, Rheumatology and Clinical Immunology, University Medical Center Ljubljana and Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Commencement: August 27th, 2020

ISSN 1024-395X
ISBN 978-9949-03-381-2 (print)
ISBN 978-9949-03-382-9 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I **Kaleviste, E.**, Saare, M., Leahy, T. R., Bondet, V., Duffy, D., Mogensen, T. H., Jørgensen, S. E., Nurm, H., Ip, W., Davies, E. G., Sauer, S., Syvänen, A. C., Milani, L., Peterson, P., and Kisand, K. (2019). Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically determined. *European Journal of Immunology*, 49(5), 790–800.

II Smyth, A. E., **Kaleviste, E.**, Snow, A., Kisand, K., McMahon, C. J., Cant, A. J., and Leahy, T. R. (2018). Aortic calcification in a patient with a gain-of-function STAT1 mutation. *Journal of Clinical Immunology*, 38(4), 468–470.

III Kasela, S., Kisand, K., Tserel, L., **Kaleviste, E.**, Remm, A., Fischer, K., Esko, T., Westra, H. J., Fairfax, B. P., Makino, S., Knight, J. C., Franke, L., Metspalu, A., Peterson, P., and Milani, L. (2017). Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. *PLoS Genetics*, 13(3), e1006643.

IV **Kaleviste, E.**, Rühlemann, M., Kärner, J., Haljasmägi, L., Tserel, L., Org, E., Trebušak Podkrajšek, K., Battelino, T., Bang, C., Franke, A., Peterson, P., and Kisand, K. (2020). IL-22 paucity in APECED is associated with mucosal and microbial alterations in oral cavity. *Frontiers in Immunology*, 11, 838.

Contributions of Epp Kaleviste to original publications:

Paper I Performed the experiments and the data analysis, prepared the figures, and wrote the paper.

Paper II Performed the flow cytometry experiment and the data analysis, participated in corresponding figure preparation and corresponding data analysis.

Paper III Performed the stimulation experiments of the mutant and wild-type alleles and the corresponding qRT-PCR analysis.

Paper IV Performed the experiments and the data analysis, prepared the figures, and wrote the paper.

ABBREVIATIONS

γδ	gamma delta
ACT1	activator 1
AD	Addison's disease
AHR	aryl hydrocarbon receptor
AIRE	autoimmune regulator
AMP	antimicrobial peptide
AP-1	activator protein 1
APECED	autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
AR	autosomal recessive
ASV	amplicon sequence variant
BP	biological process
CARD9	caspase recruitment domain-containing protein 9
CCD	coiled-coil domain
C/EBP	CCAAT/enhancer-binding protein
CFU	colony forming unit
ChIP	chromatin immunoprecipitation
ChIP-seq	chromatin immunoprecipitation coupled with high-throughput sequencing
CIITA	class II major histocompatibility complex transactivator
CMC	chronic mucocutaneous candidiasis
CXCL	CXC motif chemokine ligand
DBD	DNA-binding domain
DC	dendritic cell
EAE	experimental autoimmune encephalitis
EBI3	Epstein-Barr virus-induced gene 3
ERK	extracellular signal-regulated kinase
FACS	fluorescence-activated cell sorting
FLU	fludarabine
GAS	gamma-activated site
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GO	gene ontology
GOF	gain-of-function
GREAT	Genomic Regions Enrichment of Annotations Tool
GWAS	genome-wide association study
H3K4me3	trimethylation of lysine 4 of histone 3
HDAC	histone deacetylase
HP	hypoparathyroidism
HSCT	hematopoietic stem cell transplantation
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule-1

IFN	interferon
IFNAR	interferon-alpha/beta receptor
IKK	inhibitor of NF-κB kinase
IL	interleukin
IL-10Rβ	interleukin-10 receptor β-chain
IL-17R	interleukin-17 receptor
IL-21R	interleukin-21 receptor
IL-22RA1	interleukin-22 receptor A1
ILC	innate lymphoid cell
iNKT	invariant natural killer T
IPA	Ingenuity Pathway Analysis
IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked
IRF9	interferon-regulatory factor 9
ISG	interferon-stimulated gene
ISGF3	interferon-stimulated gene factor 3
ISRE	interferon-stimulated response element
JAK1	Janus kinase 1
JAK-STAT	Janus kinase and signal transducer of activator of transcription signaling pathway
JNK	JUN N-terminal kinase
LCN	lipocalin
LIPS	luciferase based immunoprecipitation system
LOF	loss-of-function
LU	luminescence unit
MAIT	mucosal associated invariant T
MAPK	mitogen-activated protein kinase
MFI	mean fluorescence intensity
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa B
NK	natural killer
NKT	natural killer T
NOD	non-obese diabetic
NS	unstimulated
PAMP	pathogen-associated molecular pattern
PBMC	peripheral blood mononuclear cell
PD-L1	programmed death-ligand 1
PIAS	protein inhibitors of activated Stat
PID	primary immune deficiency
PMN	polymorphonuclear neutrophil
PRR	pattern recognition receptor
pSS	primary Sjögren's syndrome
p-STAT1/3	phosphorylated STAT1 or 3
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RA	rheumatoid arthritis
ROR	retinoic acid-related orphan receptor

SD	standard deviation
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
SH2	Src homology 2
SOCS1/3	suppressors of cytokine signaling 1 or 3
STAT1/*Stat1*	signal transducer and activator of transcription 1, gene designation in human/mouse
STAT1	signal transducer and activator of transcription 1, protein designation in human and in mouse
T1D	type 1 diabetes
TFIIH	transcription factor IIH
Th	T helper
TLR	Toll-like receptor
TNF	tumor necrosis factor
TRAF6	TNF receptor associated factor 6
Treg	regulatory T
TSS	transcription start site
TssAFlnk	flanking active TSS
TYK2	tyrosine kinase 2

1. INTRODUCTION

Today, the term cytokine has acquired an intriguing and important image. Although it is vital for immune protection, cytokine overproduction can be fatal in the form of a cytokine storm, as the world has witnessed during the SARS-CoV-2 pandemics. Previous research on cytokines has also identified them as useful drug targets in several autoimmune diseases, which has led to the development of effective biological treatment regimens. Moreover, monogenic diseases with immune dysregulation may serve as a unique model for distinguishing various important nuances in cytokine function.

This study focuses on two monogenic diseases that are both characterized by the early onset of chronic mucocutaneous candidiasis (CMC) and impaired cytokine function. One is caused by a dominant gain-of-function (GOF) variant in the signal transducer and activator of transcription 1 (*STAT1*) gene and the other by an autosomal recessive pathogenic variant in the autoimmune regulator (*AIRE*) gene, leading to the syndrome called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). The diseases share intriguing similarities, such as susceptibility to candidiasis, the shortage of various T helper (Th)17 cytokines, and autoimmunity. Derived from opposing features, APECED patients have impaired type I interferon (IFN) responses due to highly neutralizing IFN-α autoantibodies, while patients with the *STAT1* GOF disease causing variant are likely suffering from exaggerated responses from IFNs.

Type I IFNs are known for their essential role in protection against viral infections and for signaling via STAT1. Nevertheless, their excessive production potentiates different pathologies, including vascular abnormalities and autoimmunity. The molecular pathways that predispose *STAT1* GOF patients for autoimmunity are unknown, as are the mechanisms leading to impaired STAT3 signaling causing Th17 cell deficiency and CMC.

Interleukin (IL)-27 is another cytokine that signals through STAT1. It has a dual role in immune regulation, as IL-27 has been characterized to have both pro- and anti-inflammatory effects. IL-27 has been suggested as a therapeutic option for several autoimmune diseases, therefore further studies about the impact of this cytokine are necessary.

IL-22 is crucial for the protection of epithelial tissues by upregulating protective antimicrobial peptides (AMPs) and securing mucosal barrier surveillance. Earlier studies have shown that APECED patients suffer from CMC due to neutralizing autoantibodies against IL-22 and IL-17F. However, whether an IL-22 shortage creates any other disturbances in APECED mucosal surfaces remains unknown.

The general aim of the thesis was to find the pathological mechanisms in two monogenic diseases by investigating the role of cytokines in the human immune system. The purpose was to clarify the role of several STAT1 and STAT3 signaling cytokines in the setting of monogenic diseases or genetic variation. We explored lymphocyte responsiveness to IFN-α and IL-21 in *STAT1* GOF patients. In addition, we evaluated the bioactivity of IL-27 coded by a missense variant of the gene that is associated with protection against type I diabetes. Furthermore, we investigated if the IL-22 paucity in APECED patients can trigger alterations in their oral mucosa other than CMC.

2. REVIEW OF THE LITERATURE

2.1. Type I IFNs and cytokines using STAT1 signaling pathway

2.1.1. Overview of interferons

IFNs are widely expressed cytokines that have potent antiviral and growth-inhibitory effects. The IFN family includes three main classes of related cytokines – type I IFNs, type II IFN, and type III IFNs (Gibbert et al., 2013; Hoffmann et al., 2015; Kotenko et al., 2003; Schroder et al., 2004). Human type I IFNs include 13 similar IFN-α subtypes (IFN-α1, α2, α4, α5, α6, α7, α8, α10, α13, α14, α16, α17, and α21), and single IFN-β, IFN-κ, IFN-ε and IFN-ω (Gibbert et al., 2013). Type II IFN, IFN-γ, is produced mainly by immune cells (Schroder et al., 2004). The type III IFNs include IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4 (Kotenko et al., 2003) (Fig. 1).

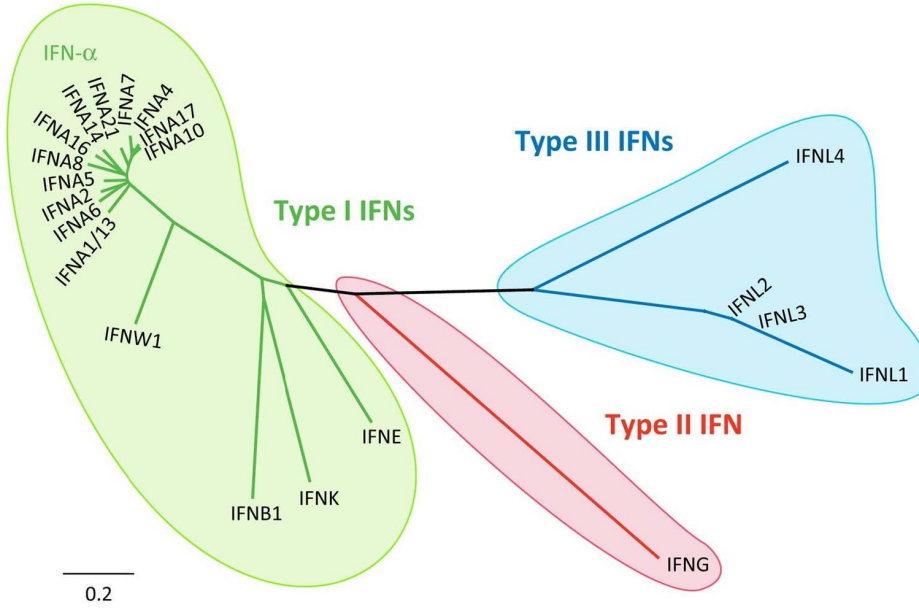

Figure 1. A phylogenetic tree of human IFNs. IFN proteins are divided into type I (green), II (red), and III (blue). The scale bar is indicating the amino acid substitutions per site. IFNA, interferon alpha; IFNW, interferon omega; IFNB, interferon beta; IFNK, interferon kappa; IFNE, interferon epsilon; IFNG, interferon gamma; IFNL, interferon lambda (Hoffmann et al., 2015).

2.1.2. Type I IFN subtypes

The current thesis focuses on type I IFNs, mainly IFN-α. All type I IFNs bind the same interferon-alpha/beta receptor (IFNAR), but they still slightly differ in their biological activities. The binding affinity between each type I IFN varies and there are differences in the tissue-specific expression of the IFN-α subtypes (Moll et al., 2011). Different viruses and the IFN-producing cell type may also change the action of IFN-α subtypes (Easlick et al., 2010). Individual IFN-α subtypes are able to induce a specific pattern of the IFN-stimulated genes (ISGs) expression, which is consistent with the cell type-specific responses and receptor affinities (Gibbert et al., 2013; Severa, 2006). From all of the type I IFN subtypes, IFN-β exhibits the strongest interaction with its receptor (Ng et al., 2016). Only IFN-β is known to induce negative immune regulators IL-10 and programmed death-ligand 1 (PD-L1) (Saraiva & O'Garra, 2010; Sharpe et al., 2007). Type I IFNs induce an antiviral state in cells by suppressing mechanisms for viral replication, trigger apoptosis in virally infected cells, promote natural killer (NK) cell-mediated and CD8+ T-cell-mediated cytotoxicity, promote the transition from innate to acquired immunity by augmenting dendritic cell (DC) maturation, cross-presentation of antigens and migration, and enhance Th1 responses, generation of T follicular helper cells, and humoral responses (Brassard et al., 2002; Cucak et al., 2009; Stetson & Medzhitov, 2006). When IFN-α or IFN-β binds to interferon receptors on circulating NK cells, these are activated and directed to infected tissues, where they attack virus-infected cells by producing perforin and granzyme and by secreting IFN-γ (Mandal & Viswanathan, 2015). In contrast, type II IFN (IFN-γ) is an activator of macrophages and does not produce any antiviral effect (Green et al., 2017).

2.1.3. Type I IFNs induction

Type I IFNs can be induced in most cell types against acute viral infections but also by several bacterial infections and nucleic acids from the host (Trinchieri, 2010). Cells sensing an infection express a number of pattern recognition receptors (PRRs) that recognize a wide array of highly conserved pathogen-associated molecular patterns (PAMPs) (Barrat et al., 2016). The PRRs recognize specific viral and bacterial-derived components (nucleic acids or membrane-bound Toll-like receptors (TLRs)), and initiate a response that results in the production of type I IFNs (Kawasaki et al., 2011). The TLRs involved in type I IFN production are TLR3 (dsRNA), TLR7/8 (ssRNA), and TLR9 (dsDNA-containing unmethylated CpG motifs and RNA-DNA hybrids), which are mainly present in immune cells (Kawai & Akira, 2010). Nearly all cells are capable of detecting PAMPs and producing IFN-β. IFN-α is mainly produced by plasmacytoid dendritic cells and monocytes/macrophages (Swiecki & Colonna, 2015).

2.1.4. Type I IFN receptor and signaling pathways

Type I IFNs bind specific cell-surface receptor IFNAR and signal via pathways using the STAT proteins and protein tyrosine kinases to activate target gene expression (Ivashkiv, 2018; Stark & Darnell, 2012; Villarino et al., 2017) (Fig. 2). IFNAR is composed of two subunits, IFNAR1 and IFNAR2, that are associated with tyrosine kinase 2 (TYK2) and Janus activated kinase 1 (JAK1), respectively (Ivashkiv, 2018; Stark & Darnell, 2012; Villarino et al., 2017). There are seven members of the STAT family mediating the transduction of signal from a vast variety of growth factors and cytokines (Villarino et al., 2017). Activation of kinases that are associated with the type I IFN receptor results in phosphorylation of STAT1 and STAT2 dimer formation. STAT1 is phosphorylated on the tyrosine residue at position 701. Together, with the IFN-regulatory factor 9 (IRF9) transcription factor, the proteins form a complex named interferon-stimulated gene factor 3 (ISGF3). The complex is translocated into the nucleus, where ISGF3 binds DNA elements termed interferon-sensitive response element (ISREs) and activates ISGs (Levy & Darnell, 2002). Type I IFNs also induce the formation of STAT1 homodimer, STAT3 homodimer, and STAT1-STAT3 heterodimer, which bind a gamma-activated site (GAS) and directly activates a specific set of ISGs (Delgoffe & Vignali, 2013) (Fig. 2). After the transcription of the target sites, STAT dimer is dephosphorylated and exported from the nucleus (Levy & Darnell, 2002).

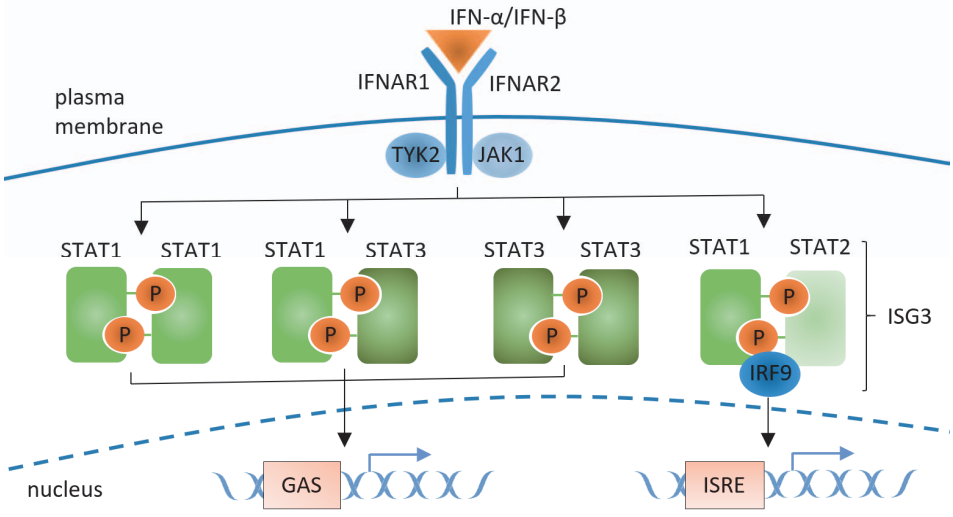

Figure 2. Type I IFN signaling pathway. On engagement, IFNAR (IFNAR1 and IFNAR2) activates JAK1 and TYK2. The receptor phosphorylation by these kinases results in STAT proteins phosphorylation, dimerization, and nuclear translocation. ISGF3 complex (STAT1, STAT2, and IRF9) binds to ISRE sequences, whereas STAT1 and STAT3 dimers bind to GASs.

In addition to JAK-STAT signaling, several other non-canonical pathways are known to be induced by type I IFNs, for example, nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) can be activated by IFN-α/β (David, 2002). Moreover, different molecules can regulate the activity of the signaling pathways. IFN-γ and IFN-α/β are negatively regulated by two groups of protein families, suppressors of cytokine signaling (SOCS) and protein inhibitors of activated Stat (PIAS) (David, 2002). Type I IFNs can induce SOCS1 and SOCS3 expression through STAT1 and STAT3 signaling pathways, respectively (Qin et al., 2008). SOCS1 inhibits JAK1, JAK2, and TYK2 and nuclear translocation of STAT1 in response to IFNs (Liau et al., 2018). SOCS1 has a stronger inhibitory activity toward STAT1 activation compared to SOCS3 (Song & Shuai, 1998). PIAS regulates JAK-STAT signaling negatively by inhibiting the phosphorylation and translocation of STAT through the interaction between PIAS and STAT (Liu et al., 1998).

2.1.5. IL-21

Besides IFNs, STAT1 is activated by other cytokines, like IL-21 (Delgoffe & Vignali, 2013) and IL-27 (Villarino et al., 2004). IL-21 signals via a receptor composed of a common cytokine receptor γ-chain and IL-21 receptor (IL-21R). IL-21 is primarily produced by CD4+ T cells and natural killer T (NKT) cells, but other cells are also able to synthesize IL-21, including CD8+ T cells, B cells, NK cells and dendritic cells (Spolski & Leonard, 2008). IL-21 binding to its cognate receptor results in the activation of JAK1 and JAK3 and the subsequent phosphorylation of STAT proteins. This concludes with the formation of STAT1 homodimer, STAT3 homodimer, and heterodimer, but the STAT3 homodimer formation is prevalent (Delgoffe & Vignali, 2013). IL-21 signaling through STAT3 is indispensable for the generation of T follicular helper cells (Nurieva et al., 2008). IL-21 contributes to the Th17 differentiation and Th17 cells produce IL-21 via STAT3 (Wei et al., 2007). STAT1 and STAT3 have partially opposing roles in IL-21 function in immune cells. IL-21 induces STAT1 phosphorylation, which is augmented in Stat3-deficient CD4+ T cells (Wan et al., 2015). IL-21 induces the expression of *Tbx21* and *Ifng* genes and ISGs, through the STAT1 transcription factor. Whereas, STAT3 activation is able to diminish these effects, either in mice or humans (Wan et al., 2015).

2.1.6. IL-27

IL-27 is a heterodimeric cytokine that contains Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28 subunits. The cytokine is produced by monocytes, dendritic cells, and endothelial cells (Villarino et al., 2004). IL-27 induces signaling through the receptor composed of gp130 and IL-27 receptor α. The receptor has been found on various cell types such as T cell subsets, NK cells,

NKT cells, dendritic cells, B cells, and mast cells (Pflanz et al., 2002, 2004). IL-27 displays both anti- and pro-inflammatory effects. IL-27 signaling results in the recruitment of several JAK family kinases and activation of STAT family transcription factors, including STAT1 and STAT3 (Villarino et al., 2004). IL-27 drives inflammation by supporting the early commitment of naïve CD4+ T cells to a Th1-specific lineage via STAT1 (Lucas et al., 2003). In addition, by suppressing Th17 differentiation, IL-27 is able to inhibit the inflammation and induce a T regulatory (Treg)-like activity in differentiated Th1 and Th2 effector cells (Delgoffe et al., 2011). Studies with *Stat1* knockout mice have shown that IL-27 loses its ability to inhibit Th17 differentiation without STAT1 (Neufert et al., 2007; Peters et al., 2015), suggesting that p-STAT1 is an inhibitor of the Th17 differentiation pathway. There have been studies associating single nucleotide polymorphisms (SNPs) of IL-27 with type 1 diabetes (T1D). A genome-wide association study (GWAS) identified a variant rs4788084[T] on chr16p11.2 close to the *IL27* gene, which is associated with protection against T1D (Barrett et al., 2009; Plagnol et al., 2011). A study by Wang et al. detected a high level of IL-27 in non-obese diabetic (NOD) mice. IL-27-treated diabetic splenocytes promoted the onset of the disease, while the blockade of IL-27 delayed the onset of diabetes (Wang et al., 2008). In addition, another study on mice showed that IL-27-deficient NOD mice were resistant to T1D (Ciecko et al., 2019).

2.2. Th17 cytokines

2.2.1. Th17 cell differentiation

Th17 cells mediate immune protection against extracellular pathogens – fungi and bacteria (LeibundGut-Landmann et al., 2007). The tissue and microbial microenvironment are known to influence Th17 immune responses, for example, commensal microbiota of the gut (Ivanov et al., 2008; Satoh-Takayama et al., 2008) and microbial stimuli on the skin induce Th17 cell differentiation and modulate Th17 cell responses (K. Eyerich et al., 2009). The differentiation of Th17 cells takes place following the exposure of naive CD4+ T cells to APC-derived polarizing cytokines such as transforming growth factor beta (TGF-β), IL-6, IL-21, while IL-23 stabilizes the commitment of Th17 cell lineage (Korn et al., 2009). The main function of IL-23 is to maintain the Th17 cell phenotype and survival, not to promote the differentiation (Lee et al., 2012). STAT3 is indispensable for the development of Th17 cells and has a crucial role in protection against mucocutaneous fungal diseases (Puel et al., 2012). The master regulators of Th17 differentiation are retinoic acid-related orphan receptor (ROR)γt and RORα transcription factors (Ivanov et al., 2006; Yang et al., 2008). Internal microenvironmental stimuli are involved in the differentiation of naïve T cells into effector T cells. TGF-β and IL-2 enhance the differentiation of Tregs, whereas the combination of TGF-β, IL-6, and IL-21 polarizes T cells towards the Th17 phenotype (Burgler et al., 2009; Kryczek et al., 2007; Zheng et al., 2008). The

decision between regulatory and pro-inflammatory outcome is a balancing act with the microenvironment being an important mediator of tissue homeostasis. Cytokines IFN-α/β, IFN-γ, and IL-27 inhibit Th17 cell differentiation, acting through the transcription factor STAT1 (Shuai & Liu, 2003). Fabbri et al. hypothesized that STAT1 affects Th17 development via a T-cell intrinsic and extrinsic mechanism (Fabbri et al., 2019). In Th1 conditions, STAT1 induces SOCS3 upon IFN-γ stimulation. SOCS3, in turn, inhibits STAT3 activity and consequently suppresses Th17 development (Yoshimura et al., 2012).

2.2.2. Th17-related cytokines and their producers

Th17 cells produce the cytokines IL17 (IL-17A and IL-17F), IL-21, and IL-22 (Burgler et al., 2009; Wei et al., 2007). IL-17 and IL-22 are derived from adaptive and innate immune cells. The best characterized human leukocytes that secrete both IL-17 and IL-22 are Th17 cells (Burgler et al., 2009). In addition, several other cell types contribute to IL-17 and IL-22 production, including CD4+ (Rutz et al., 2014), CD8+ (Ortega et al., 2009), as well as type 3 innate lymphoid cells (Cella et al., 2009) and NK cells (Pandya et al., 2011). Furthermore, IL-17 and IL-22 are secreted by several unconventional T cells, such as gamma delta (γδ) T (Ness-Schwickerath & Morita, 2011), mucosal associated invariant T (MAIT) (Leeansyah et al., 2014), NKT (Witte et al., 2010), and invariant NKT (iNKT) cells (Paget et al., 2012). Unconventional T cells are crucial for the protection and homeostasis of the epithelial surfaces due to their immediate response to harmful agents (Veldhoen et al., 2008). In addition, two cell lineages produce only IL-22, such as the NK22 cells (Norian et al., 2009) and Th22 cells (S. Eyerich et al., 2009). NK22 cells are enriched in the gastrointestinal system, where they protect against infection (Norian et al., 2009). Th22 cells are enriched in inflammatory skin diseases (S. Eyerich et al., 2009).

2.2.3. IL-17A/F signaling pathways and receptors

IL-17A and IL-17F function either as homodimers or as a heterodimer, which induce signals through an IL-17 receptor (IL-17R)A and IL-17RC receptor complex (Gaffen, 2009) (Fig. 3). IL-17-dependent signaling pathways all require an adaptor protein activator 1 (Act1) for the activation (Gu et al., 2013). Act1 recruits and ubiquitinates tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6), which is required for the activation of several pathways, such as NF-κB, MAPKs, and also CCAAT/enhancer-binding protein (C/EBP) signaling (Swaidani et al., 2019). Very recently it has been shown that in addition to the classical IL-17RA-dependent signaling, IL-17F homodimer is able to form a complex only with receptor IL-17RC, thus competing with IL-17RA for cytokine binding (Goepfert et al., 2020).

TRAF6 provides a scaffold for the recruitment and activation of the inhibitor of NF-κB kinase (IKK) (Amatya et al., 2017). IKK then phosphorylates and activates NF-κB, releasing it for a rapid nuclear translocation and consequent inflammatory gene transcription (Napetschnig & Wu, 2013) (Fig. 3). IL-17 also activates MAPK pathways, which include extracellular signal-regulated kinase (ERK), p38, and JUN N-terminal kinase (JNK) (Fig. 3), although the dominance of these pathways in response to IL-17 appears to vary (Shen & Gaffen, 2008). C/EBP transcription factors are additional transcriptional regulators activated by IL-17 (Fig. 3). In the promoters of IL-17 target genes both NF-κB and C/EBP binding sites are abundant (Shen et al., 2006).

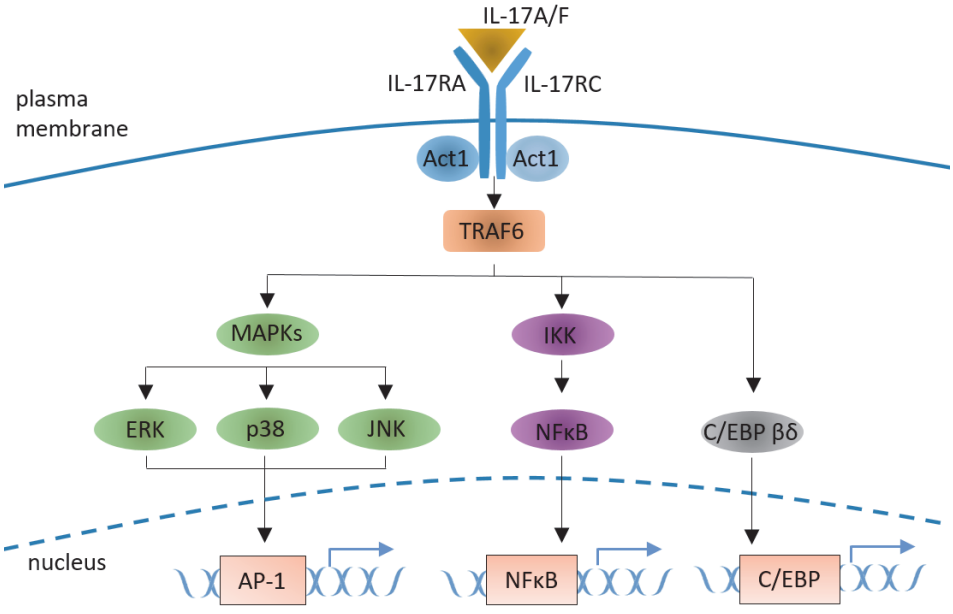

Figure 3. Intracellular signaling pathways of IL-17A and IL-17F. IL-17A and IL-17F bind to the IL-17RA or IL-17RC as homodimers or heterodimers. Binding of IL-17A to its receptor complex leads to the recruitment of the Act1 adaptor protein. Association of Act1 with the IL-17 receptor complex leads to the recruitment of TRAF-6. Activation of TRAF6 results in the triggering of NF-κB, C/EBPβ, C/EBPδ, and MAPK pathways. TRAF6, TNF receptor associated factor 6; ERK, extracellular signal related kinase; JNK, JUN N-terminal kinase; AP-1, activator protein 1; IKK, inhibitor of NF-kappa B kinase; CCAAT/enhancer-binding protein β or δ, C/EBP βδ.

2.2.4. Role of IL-17A/F

The effects of IL-17 and IL-22 are organ-specific, and their function should be considered in the context of target organs. IL-17+ and IL-22+ leukocytes are more enriched in peripheral tissue than in circulation (K. Eyerich et al., 2009).

The main function of IL-17 and IL-22 is the induction of an epithelial innate immune response for protection against extracellular pathogens. The protection mechanism is the induction of AMPs production, such as β-defensins (Liang et al., 2006) and S100 proteins (Wolk et al., 2006), in epithelial cells of the lung and gastrointestinal system and skin (Norian et al., 2009). AMPs are generally defined by their ability to kill or inhibit the growth of microbes, and they are integral to the innate immune response in all multicellular organisms, representing a key aspect of barrier protection (Zhang & Gallo, 2016) (Fig. 5).

IL-17 is involved in protective antimicrobial immunity and inflammation (Fig. 5). In response to bacterial infection of lungs, IL-17 is important for the recruitment of neutrophils (Ye, Rodriguez, et al., 2001). The key neutrophil chemoattractants such as macrophage inflammatory protein-2 and granulocyte colony-stimulating factor (G-CSF) are induced by IL-17 and required for recruitment of neutrophils and pathogen clearance (Ye, Garvey, et al., 2001). Pro-inflammatory mediators produced by epithelial cells in response to IL-17 are neutrophil- and granulocyte-attracting chemokines, mainly CXC motif chemokine ligand (CXCL) 8 (Albanesi et al., 2000) and several growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF). These enhance inflammation by recruiting macrophages, neutrophils, and lymphocytes to the synovium (Albanesi et al., 2000). IL-17 also induces pro-inflammatory cytokine secretion (TNF, IL-1β, IL-6) from cartilage, macrophages, and synoviocytes (Zenobia & Hajishengallis, 2015). Interestingly, IL-17 is not able to mount a robust inflammatory response by itself, however, in cooperation with other inflammatory mediators, such as TNF-α or IL-1, IL-17 can induce a potent inflammatory cascade by upregulating the expression of target genes (Onishi & Gaffen, 2010; Zenobia & Hajishengallis, 2015). By a self-reinforcing positive feedback loop, these pro-inflammatory cytokines also establish a chronic inflammatory state (Ogura et al., 2008).

Moreover, by increasing intercellular adhesion molecule-1 (ICAM-1)-dependent cell contact of keratinocytes and T cells and subsequent keratinocyte apoptosis, IL-17 induces a strong pro-inflammatory reaction and reinforces a cytotoxic Th1 immune response (Nograles et al., 2008). IL-17 promotes antigen-nonspecific and contact-dependent cytotoxicity by enhancing IFN-γ-induced upregulation of ICAM-1 on keratinocytes (Albanesi et al., 1999). IL-17 induces the expression of matrix metalloproteinases (MMP), which drive the degradation of extracellular matrix within the joint (Chabaud et al., 2000; Onishi & Gaffen, 2010) (Fig. 5).

IL-17 mediates adverse effects in many autoimmune diseases. Studies show that high IL-17 levels were found in patients with rheumatoid arthritis (RA) (Kotake et al., 1999). IL-17 appears to promote both bone destruction and inflammation in RA (Lubberts et al., 2004). IL-17A and IL-17F play opposite roles in inflammatory bowel diseases (IBDs). Notably, IL-17F is pathogenic in IBDs inducing microbial dysbiosis of the gut microbiota by stimulating the expression of AMPs, while IL-17A plays a protective role contributing to the maintenance of the intestinal epithelial barrier (Tang et al., 2018).

2.2.5. IL-22 signaling pathways and receptors

IL-22 binds to a heterodimeric receptor composed of IL-10 receptor β-chain (IL-10Rβ) and IL-22 receptor A1 (IL-22RA1) (Rutz et al., 2014) (Fig. 4). While IL-10Rβ is broadly expressed in the human body, IL-22 responsiveness is limited by epithelial cell-restricted expression of IL-22RA1 in the lung, gastrointestinal tract, thymus, skin, pancreas, liver, and kidney (Rutz et al., 2014). In contrast to IL-17RA, IL-22 receptor is expressed exclusively on stromal cells and is absent on immune cells (Wolk et al., 2004). However, one study showed that monocyte-derived human macrophages express IL-22 receptor and IL-22 enhances the killing of tuberculosis bacteria (Dhiman et al., 2009). The bioavailability of IL-22 is also regulated by a soluble IL-22-binding protein that acts as an antagonist (Xu et al., 2001). Upon binding to its receptor complex, IL-22 induces phosphorylation of tyrosine kinases TYK2 and JAK1 (Lejeune et al., 2002), which results in activation of STAT3 and also STAT1 (Dumoutier et al., 2000) (Fig. 4). Similarly to IL-17, IL-22 induces the three major MAPK pathways – Mek/Erk, p38, and JNK (Lejeune et al., 2002) (Fig. 4).

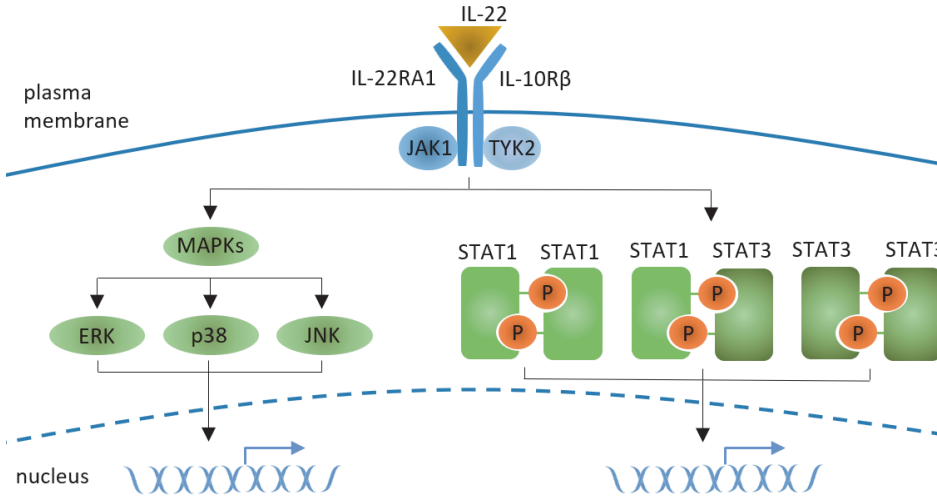

Figure 4. IL-22 binds to a heterodimer composed of IL-10Rβ-chain and IL-22RA1. Upon binding to its receptor, IL-22 induces phosphorylation of tyrosine kinases TYK2 and JAK1 that activate transcription factors STAT1 and/or STAT3. Phosphorylated STAT dimers translocate into the nucleus to induce the expression of specific genes. In some cases, MAPKs (ERK, p38, and JNK) are also activated through a distinct pathway.

2.2.6. Role of IL-22

IL-22 is essential for the mucosal barrier function. It can protect from intestinal injury by supporting epithelial cell proliferation and wound healing, enhancing tight junctions, upregulating AMPs, and mucus production (Aujla et al., 2008;

Bevins & Salzman, 2011; Boniface et al., 2005; Sugimoto et al., 2008) (Fig. 5). In contrast, the excessive production of IL-22 is associated with tissue inflammation in several immune-mediated inflammatory diseases, such as psoriasis, celiac disease, and RA (DePaolo et al., 2011; Geboes et al., 2009; Šahmatova et al., 2017; Zheng et al., 2007).

In mucosal epithelial cells, IL-22 induces the expression of mucin genes through STAT3-dependent signaling (Sugimoto et al., 2008). In addition, IL-22 treatment increases the number of goblet cells in the intestinal mucosa, which produce mucin in the colon (Turner et al., 2013). Specialized epithelial cells called Paneth cells are the major source of AMPs in the intestine, and the production of AMPs can be elicited by IL-22 (Bevins & Salzman, 2011). Moreover in other tissues, IL-22 elicits AMP production in keratinocytes (Liang et al., 2006) and pancreatic acinar cells (Liang et al., 2010). In addition, IL-22 functions in the clearance of pathogens that have managed to penetrate the barrier. For example, to facilitate the production of cytokines and chemokines that mediate innate cell recruitment to the site of infection, IL-22 can induce IL-18 expression in epithelial cells (Muñoz et al., 2015). IL-22 can also induce the complement pathway. IL-22 treatment can increase complement *C3* gene expression, to induce bacteria killing and control the systemic spread of bacteria (Hasegawa et al., 2014).

In contrast to the strong pro-inflammatory effects of IL-17, IL-22 has a regenerative and protective effect on epithelial cells (Fig. 5). In addition to the induction of AMPs, IL-22 is able to induce re-epithelialization and enhances the proliferation and migration of epithelial cells, and inhibits differentiation of keratinocytes (Boniface et al., 2005). These changes in keratinocytes resemble wound healing and/or ongoing psoriasis, which suggest that IL-22 is involved in both processes (Boniface et al., 2005; Zheng et al., 2007). Furthermore, IL-22 protects lung function by increasing transepithelial resistance to injury and promotes barrier function by induction of epithelial cell proliferation (Aujla et al., 2008). Adoptive transfer of IL-22-producing CD4+ T cell and NK cells protects mice from IBDs (Zenewicz et al., 2008) and hepatitis (Radaeva et al., 2004). Administration of recombinant IL-22 ameliorates inflammation, whereas neutralization of IL-22 aggravates hepatitis (Radaeva et al., 2004).

IL-22 upregulates anti-apoptotic as well as mitogenic proteins via STAT3 (Radaeva et al., 2004) and prevents systemic inflammation by induction of lipopolysaccharide-binding proteins in hepatocytes (Wolk et al., 2007). IL-22 is required for the activation of the DNA damage response in the intestinal epithelium. In a series of elegant experiments, Gronke et al. demonstrated that selective removal of IL-22RA1 leads to suppression of apoptosis triggered by DNA damage, and, as a consequence, increased tumor formation in an inflammation-driven tumor model (Gronke et al., 2019). In contrast, IL-22 was recently shown to protect intestinal stem cells against genotoxic stress and thus against colon cancer (Gronke et al., 2019).

IL-22 is capable of shaping gut microbiota (Fatkhullina et al., 2018). Although IL-22 shapes the microbiome and maintains the intestinal barrier and through

multiple mechanisms, the IL-22 deficiency does not result in overt pathological consequences. Nevertheless, IL-22-deficient mice have altered microbiota composition and weakened barrier function, which can be rescued with IL-22 treatment (Sonnenberg et al., 2012). IL-23 receptor-deficient animals are producing less IL-22 and they have increased segmented filamentous bacteria in the ileum, which generates a permissive environment to the development of Th17 cells. This phenotype can be reversed by the administration of IL-22 (Shih et al., 2014). There have been studies describing IL-22-deficient mice with less bacterial diversity and decreased number of *Lactobacillus*, but an increased abundance of *Salmonella*, *Escherichia*, and *Helicobacter*, that showed higher susceptibility to colitis in cohoused wild-type animals. These results indicate that IL-22-deficient animals can harbor microbiota with transferable effects on barrier function (Zenewicz et al., 2013).

Nevertheless, IL-22 can have some pro-inflammatory properties as well. Although IL-22 itself seems to be protective, it enhances the pro-inflammatory capacity of TNF-α on keratinocytes (S. Eyerich et al., 2009). Thus, IL-22 can be both protective and pro-inflammatory depending on the context of the micro-environment.

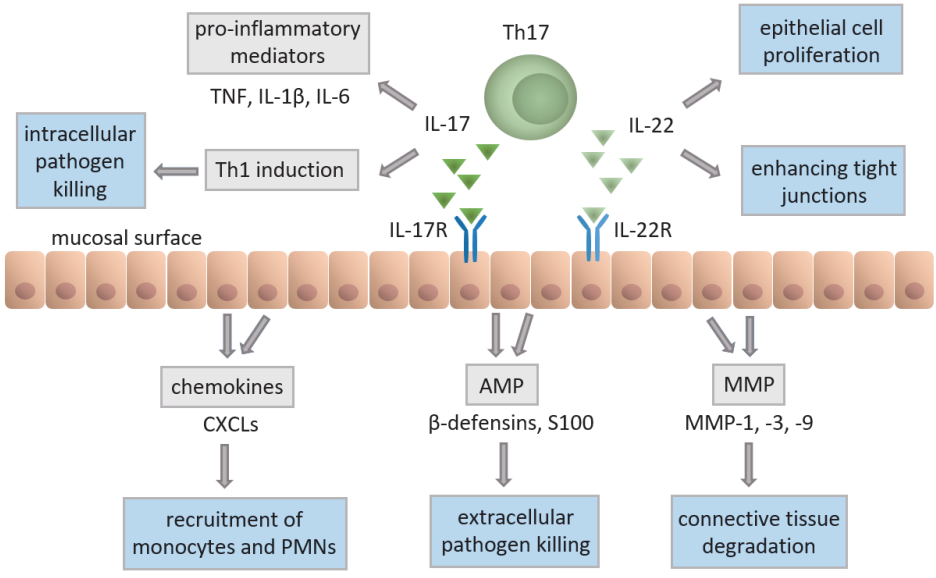

Figure 5. IL-17 and IL-22 augment chemokines production resulting in the recruitment of polymorphonuclear neutrophils (PMNs), monocytes, and other inflammatory cells that contribute to fungal, bacterial, and viral clearance at mucosal sites. IL-17 can synergize with IL-22 and induce AMPs to control the extracellular pathogens. IL-17 and IL-22 stimulate the production of MMPs to promote the destruction of connective tissue. IL-17 can also act directly on APCs, and drive Th1 differentiation required for intracellular pathogen clearance. IL-17 also induces pro-inflammatory cytokine secretion. IL-22 is involved in epithelial cell proliferation and enhances tight junctions.

2.3. Monogenic *STAT1* GOF disease causing variant

2.3.1. Chronic mucocutaneous candidiasis

The GOF variant in the *STAT1* gene is the most frequent one that leads to monogenic susceptibility to CMC (Toubiana et al., 2016). GOF variants are described in the DNA-binding domain (DBD), coiled-coil domain (CCD) and occasionally in Src homology 2 (SH2) domain in the *STAT1* gene. The pathogenic variants lead to the hyperphosphorylation and delayed dephosphorylation of STAT1 during cytokine responses (Liu et al., 2011; Meesilpavikkai et al., 2017; Smeekens et al., 2011; van de Veerdonk et al., 2011; Yamazaki et al., 2014) (Fig. 6). CMC is a recurrent and/or chronic, noninvasive *Candida* infection on the mucous membranes, skin, and nails. After the exclusion of the secondary reasons affecting the immune system such as infections (human immunodeficiency virus) or prolonged immunosuppressive drug use (glucocorticoids) primary immune deficiencies (PID) should be considered (Depner et al., 2016). CMC is a frequent component of combined immunodeficiencies with decreased T-cell numbers or function. With CMC, it is expected to have a susceptibility to various infectious agents and noninfectious manifestations, such as autoimmunity. CMC may be a single or also a coexisting infection in other PID syndromes, like APECED with autosomal recessive (AR) *AIRE* pathogenic variants (Peterson & Peltonen, 2005), AR caspase recruitment domain-containing protein 9 (CARD9) deficiency with invasive fungal diseases (Glocker et al., 2009), hyper IgE syndrome with autosomal dominant *STAT3* variants (Minegishi et al., 2007), AR IL-12 receptor-beta1 (de Beaucoudrey et al., 2010), and IL-12-p40 deficiency causing susceptibility to mycobacterial diseases (Prando et al., 2013). In some CMC patients, *IL17RC,*

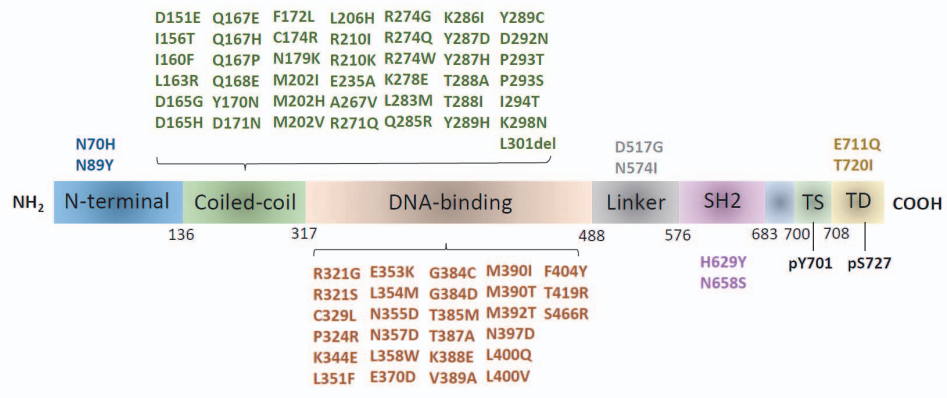

Figure 6. STAT1 domains and germline GOF *STAT1* pathogenic variants in human STAT1α isoform. GOF variants are preferentially identified in coiled-coil (green) and DNA-binding (brown) domain of STAT1. Phosphorylation sites, Tyr 701 (pY701), and Ser 727 (pS727) are indicated. SH2, Src Homology 2 domain; TS, tail segment domain; TD, transactivation domain.

IL17RA, and *IL17F* variants are found to be causative (Puel et al., 2011). The common feature of all these monogenic diseases with CMC susceptibility is the deficiency of Th17 cells and/or neutralizing autoantibodies to Th17-related cytokines. Although CMC is the most frequent infection in *STAT1* GOF disease, the patients have also bacterial and viral infections (Toubiana et al., 2016). In addition, many patients also develop autoimmune manifestations (Lorenzini et al., 2017), as well as carcinomas and aneurysms associated with the poor outcome of the disease. Autoimmune disorders described in these patients include thyroid disease, cytopenias, SLE, T1D, alopecia, and vitiligo (Toubiana et al., 2016).

2.3.2. Molecular mechanism of *STAT1* GOF disease

STAT1 GOF disease causing variant results in hyperphosphorylation and delayed dephosphorylation of STAT1 protein (Liu et al., 2011; Smeekens et al., 2011; Yamazaki et al., 2014). Why p-STAT1 dephosphorylation is delayed and how this imbalance exactly causes the increased expression of STAT1-dependent genes in *STAT1* GOF patients is not yet clear.

p-STAT1 has to be dephosphorylated for shuttling back to the cytoplasm (Levy & Darnell, 2002). During dephosphorylation to expose phospho-Tyr-701 to phosphatases, p-STAT1 requires the conversion of the STAT dimer parallel conformation to its antiparallel conformation. The antiparallel conformation of the STAT1 dimer occurs through phospho-Tyr-701-independent interaction between the DBD and CCD (Mertens et al., 2006). *STAT1* pathogenic variants have been identified mostly in DBD and CCD, residing in the antiparallel dimer interface (Liu et al., 2011; Smeekens et al., 2011; van de Veerdonk et al., 2011; Yamazaki et al., 2014). The stabilization of the antiparallel dimer conformation is likely impaired due to the amino acid change in the interacting sites (Mertens et al., 2006; Zhong et al., 2005). Nevertheless, it is unclear which residues present a risk of disease-causing variant (Fujiki et al., 2017). Direct evidence is still lacking, but it is possible that the delayed dephosphorylation of p-STAT1 is associated with its prolonged binding to the chromatin target sites. Another possible mechanism can be behind the DNA formation with CCD. The conformation of CCD protrudes outward when bound to the DNA (Chen et al., 1998; Fujiki et al., 2017). It has been postulated that, to support STAT1-mediated signaling, CCD acts as a docking platform for transcription factors. The *STAT1* GOF disease causing variants in the CCD might increase their interactions (Horvath et al., 1996).

Several studies have proposed that the possible cause behind the disease-causing variant is delayed dephosphorylation (Chen et al., 2020; Liu et al., 2011; Smeekens et al., 2011; Yamazaki et al., 2014). Controversially, in some studies, dephosphorylation has been found to be normal (Meesilpavikkai et al., 2017; Sobh et al., 2016; Weinacht et al., 2017; Zimmerman et al., 2019). In addition, previous studies have shown different results of the expression of total STAT1 in *STAT1* GOF patients. Some authors had reported normal levels of STAT1

(Mizoguchi et al., 2014; Smeekens et al., 2011; Zheng et al., 2015), while some authors had reported higher levels of STAT1 (Bernasconi et al., 2018; Bloomfield et al., 2018; Chen et al., 2020; Zhang et al., 2017). It is proposed that the high level of p-STAT1 after stimulation is not caused by defective dephosphorylation but rather by increased STAT1 protein level (Bernasconi et al., 2018; Bloomfield et al., 2018; Chen et al., 2020; Zhang et al., 2017).

Importantly, in most of the *STAT1* GOF patients, the Th17 cell numbers are severely reduced, which is thought to be the results of STAT3 inhibition by *STAT1* GOF variant. Previous results have demonstrated that *STAT1* GOF variant does not impair STAT3 phosphorylation, dimer formation, transformation to the nucleus, or DNA-binding to a STAT-consensus sequence. In fact, the results show that the imbalance disrupts the transcription of genes by increasing STAT1 but decreasing STAT3-dependent gene transcription (Zheng et al., 2015). Therefore, *STAT1* GOF variant impairs the development of Th-17 cell subpopulation (Liu et al., 2011; Takezaki et al., 2012).

2.3.3. Treatment of patients with *STAT1* GOF disease

Patients with CMC and *STAT1* GOF disease are managed by long-term administration of systemic antifungal drugs, replacement immunoglobulins, and prophylactic antibiotics (Toubiana et al., 2016). Particularly in the presence of autoimmune phenomena, the diverse phenotype of patients often renders such treatment insufficient (Toubiana et al., 2016).

STAT1 phosphorylation can be blocked in a dose-dependent manner with the nucleotide analog and STAT1 inhibitor fludarabine (FLU). It has been reported that FLU significantly decreases the transcription of STAT1-dependent genes and increases the transcription of STAT3-dependent genes (Zheng et al., 2015).

Another treatment is ruxolitinib, a JAK1/2 inhibitor that targets JAK1/2 pathway signaling and JAK-STAT pathways (Harrison et al., 2012). There have been controversial outcomes in case of ruxolitinib treatment. GOF STAT1 hyperphosphorylation has been successfully downregulated by ruxolitinib *in vitro* treatment which has encouraged its clinical application for this rare monogenic disease (Bloomfield et al., 2018; Forbes et al., 2018; Higgins et al., 2015; Moriya et al., 2020; Mössner et al., 2016; Vargas-Hernández et al., 2018; Zimmerman et al., 2019). Several reports have described improvement or resolution of CMC during ruxolitinib treatment and its return after discontinuation of the therapy (Bloomfield et al., 2018; Mössner et al., 2016). Some patients have complete resolution of oral *Candida* while some patients only have partial resolution with relieved symptoms (Higgins et al., 2015; Mössner et al., 2016). Interestingly, other *STAT1* GOF disease-related disorders, such as alopecia and enteropathy have also shown considerable improvement with JAK1/2 inhibitors (Higgins et al., 2015; Vargas-Hernández et al., 2018). In contrast to these reports, two adult patients with severe dermatophytosis and disseminated coccidioidomycosis due to *STAT1* GOF disease causing variant were recently reported to fail to respond

to ruxolitinib (Zimmerman et al., 2017). In addition, therapeutic failure of ruxolitinib in the treatment of severe fungal infections, such as CMC, or herpes zoster infections has been reported (Forbes et al., 2018).

It has been suggested that the reduced STAT3-dependent gene transcription is likely due to altered STAT3 promoter binding precipitated by reduced baseline level of histone acetylation of STAT3 in patients (Zheng et al., 2015). More recently several histone deacetylases (HDAC) inhibitors have been under investigation (Rösler et al., 2018). HDAC inhibitors modulate cytokine production in response to *C. albicans* and *S. aureus in vitro*. Pan-inhibitors lower overall cytokine production, whereas specific inhibitors confer a selective effect (Rösler et al., 2018). Some of HDAC inhibitors are promising therapeutic candidates for the treatment of *STAT1* GOF patients due to their capacity to restore IL-22 production and decrease STAT1 phosphorylation; however, their inhibition of innate cytokines poses a possible risk of secondary infections (Rösler et al., 2018).

In addition, a small number of patients have been treated with hematopoietic stem cell transplantation (HSCT) with mixed outcomes. Therefore, the recommendation for this therapy is inconclusive (Aldave et al., 2013; Kiykim et al., 2019; Leiding et al., 2018; Toubiana et al., 2016). The symptoms associated with *STAT1* GOF disease causing variant disappear after HSCT, suggesting its curative potential. However, HSCT has a significant risk of secondary graft failure (50% of patients with primary engraftment) and death. After transplantation, the 3-year overall survival rate is 40% (Leiding et al., 2018).

2.4. Type I IFNs in polygenic autoimmunity

The elevated production of IFNs during autoimmune diseases and infection results in increased expression of target genes, mostly ISGs. The pattern of expression is defined as an IFN signature in diseased tissues and in circulating blood cells (Platanias, 2005). ISGs are usually directly activated by the STAT family (Platanias, 2005). The upregulated type I IFNs and IFN gene signatures are associated with sporadic autoinflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE) (Rodero et al., 2017), T1D (Ferreira et al., 2014), primary Sjögren's syndrome (pSS) (Båve et al., 2005), systemic sclerosis (Tan et al., 2006), and in some cases of RA (Banchereau et al., 2004).

SLE has been described as an interferonopathy, in which patients exhibit an IFN gene signature, which is overlapping with T1D (Jean-Baptiste et al. 2017). Also, SLE patients show activated JAK-STAT pathway with elevated levels of STAT1 protein (Liang et al., 2014). For that reason, several therapeutic strategies exist to downregulate the type I IFN system in SLE. IFN-α or IFNAR therapeutic targeting has shown some promising results (Jean-Baptiste et al., 2017). Also, patients with pSS have activation of the type I IFN system, nevertheless circulating IFN-α levels were consistently elevated in some studies but not in others, in contrast to findings in SLE (Båve et al., 2005). There have been several reports of systemic sclerosis induced by IFN-α therapy for hepatitis C or

myeloproliferative diseases, or by IFNβ therapy for multiple sclerosis. Patients with systemic sclerosis showed amplification of mRNAs for a few genes involved in the IFN pathway (Tan et al., 2006), although the IFN signature was less typical than in SLE. The new small-molecule JAK inhibitors (Jakinibs) now in use for the treatment of RA may prove useful in SLE because type I IFNs as well as several other cytokines signal through the JAK-STAT pathway. Based on preclinical data, there are now trials probing the efficacy of Jakinibs in the treatment of diseases characterized by an IFN signature such as lupus (Furumoto et al., 2017).

T1D is a multifactorial disease caused by autoimmune destruction of pancreatic β-cells. Autoreactive CD4+ and CD8+ T cells are considered as the primary drivers of β-cell loss (Burrack et al., 2017), and among CD4+ T cells, Th1 and Th17 cells are considered the main effector subsets involved in the disease pathogenesis (Shao et al., 2012). Type I IFNs have been implicated in the early stages of T1D autoimmunity (Ferreira et al., 2014; Huang et al., 1995), as the transcriptome analysis revealed that prior to the development of T1D-specific autoantibodies there is a type I IFN signature in the peripheral blood of patients (Ferreira et al., 2014). Moreover, in patients receiving type I IFN therapy for various conditions including hepatitis C and multiple sclerosis induction of T1D has been reported (Fabris et al., 2003; Oka et al., 2011; Uonaga et al., 2012), supporting the idea that these cytokines may actively exacerbate T1D development. Following IFN therapy half of all cases reporting T1D were positive for autoantibodies against T1D. Therefore, type I IFNs may precipitate a loss of tolerance and self-reactivity in at-risk patients (Fabris et al., 2003). Type I IFNs have an impact on pancreatic β-cell, cytotoxic T-lymphocytes, and other cellular constituents within the islet that facilitate autoimmunity. Patients with T1D exhibit hyperexpression of MHC-I in the islets, suggesting increased susceptibility for targeting by cytotoxic T cells (Willcox et al., 2009). As IFN-α/β has been shown to directly induce MHC expression on primary human islet cells, type I IFN is a likely candidate within the local microenvironment capable of mediating this cytotoxicity (Marroqui et al., 2017).

2.5. APECED

2.5.1. APECED phenotype

APECED syndrome is a rare recessive disorder (Kisand & Peterson, 2015). APECED is caused by loss-of-function variants in the *AIRE* gene located in locus 21q22.3 (Peterson et al., 2008). *AIRE* is expressed in thymic medullary epithelial cells where it acts as a transcriptional regulator and promotes the ectopic expression of several peripheral tissue-restricted antigens, which leads to the deletion of potentially autoreactive T-cells (Anderson et al., 2002; Heino et al., 1999; S. Yang et al., 2015). The most common *AIRE* pathogenic variant affects 82% of Finnish APECED alleles, which is the "Finnish variant," (c. 769C>T, p.Arg257Ter) (Kisand & Peterson, 2015). Also, in exon 8 a 13-base pair deletion

(c.967–979del) is common (Nagamine et al., 1997; Peterson et al., 2004). Whilst the APECED clinical phenotype is variable, no strong correlations with specific *AIRE* variants have been reported (Halonen et al., 2002). APECED is spread worldwide but specifically enriched in Iranian Jews (1:9 000) (Zlotogora & Shapiro, 1992), Sardinians (1:14 000) (Rosatelli et al., 1998), and Finns (1:25 000) (Perheentupa, 2006). The prevalence has also been reported for Slovenia (1:43 000) (Podkrajšek et al., 2005), Norway (1:80 000) (Myhre et al., 2001) and Poland (1:129 000) (Stolarski et al., 2006).

Although the classical triad of APECED consists of CMC, hypoparathyroidism (HP), and Addison's disease (AD), the clinical picture varies broadly in the number of disease components and severity (Fig. 7). The syndrome is characterized by a variety of associations with autoimmune non-endocrine (vitiligo, alopecia areata, keratitis, malabsorption) and endocrine (HP, AD, hypothyroidism, gonadal insufficiency, T1D) disorders, and predisposition to CMC (Husebye et al., 2009; Perheentupa, 2006; Peterson & Peltonen, 2005). Juvenile APECED patients are diagnosed with vitiligo (8–25%) and alopecia (16–40%) more often than in the adult type with 5–10 and 3–10%, respectively (Betterle et al., 1998). Autoimmune hepatitis is often referred to as a rare component of APECED and is detected in 10–20% of the Finnish and in 20–30% of the Sardinian populations, respectively. Further T1D occurs in about 10–20% Juvenile patients (Husebye et al., 2009). Sjögren syndrome occurs frequently among patients in the United States (Ferre et al., 2016). Nevertheless, in European APECED patients Sjögren syndrome is rare, and is reportedly present only in adult patients (Oftedal et al., 2017). The complete triad develops in about two-thirds of the patients, and additional clinical manifestations can present for nearly the entire lifespan (Ahonen et al., 1990). The order of the main symptoms occurring is relatively consistent (Ahonen et al., 1990; Husebye et al., 2009; Perheentupa, 2006). CMC is the first sign of APECED in 75–93% of cases and usually appears already in infancy (Ahonen et al., 1990; Husebye et al., 2009; Perheentupa, 2006). This is followed by HP (peak between 4 and 5 years of age), and then by AD, which also usually appears in childhood (Ahonen et al., 1990; Husebye et al., 2009).

APECED should be a suspicion if there is a sign of CMC onset as oral thrush or recurrent napkin dermatitis (Ahonen et al., 1990; Husebye et al., 2009). In APECED patients the susceptibility to candidiasis maps to mucosal, not systemic disease (Perheentupa, 2006). The severity and course of candidiasis vary between patients, it can be a mild and remittent infection or in more severe cases may lead to the development of chronic hypertrophic and/or atrophic lesions. CMC tends to become milder and is self-treatable in adulthood (Ahonen et al., 1990; Husebye et al., 2009). Because oral and esophageal candidiasis are associated with the high risk of the development of squamous cell carcinoma, the infection should be carefully controlled with antifungal treatment (Rautemaa et al., 2007).

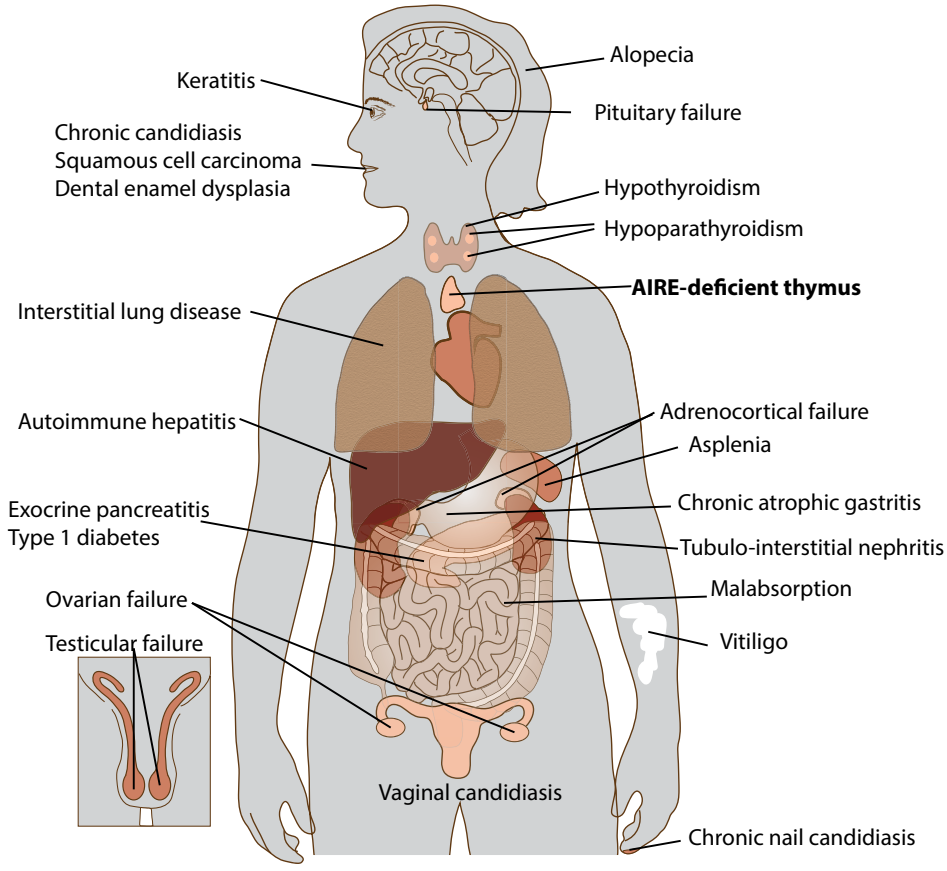

Figure 7. The classical triad of APECED consists of CMC, HP, and AD, nevertheless, the clinical picture varies broadly in severity and the number of disease components, with up to ten disorders per patient (Kisand & Peterson, 2015).

2.5.2. Autoantibodies in APECED

APECED patients have a diverse set of autoantibodies in high titers, mostly against tissues affected by the disease (Fishman et al., 2017; Kisand & Peterson, 2015; Peterson & Peltonen, 2005). Many autoantibodies against organ-specific antigens associated with APECED recognize intracellular enzymes involved in neurotransmitter or hormone biosynthesis (Husebye & Anderson, 2010).

An outstanding feature of APECED is that all patients develop high titers of neutralizing autoantibodies against type I IFNs already as early as in few months (Kisand et al., 2008; Wolff et al., 2013). These type I IFN autoantibodies have become diagnostic or also prognostic markers for APECED patients (Meager et al., 2006). The autoantibodies against type I IFNs have been shown to down-regulate the expression of interferon-controlled genes (Kisand et al., 2008; Wolff et al., 2013). In medullary thymic epithelial cells, type I IFNs are not regulated

by AIRE, and therefore the autoimmune reaction towards them cannot be explained by an impaired negative selection in the APECED thymus. Low-titer autoantibodies to type I IFNs are also detected in diseases that are accompanied by increased IFN production (genetic interferonopathies, SLE). However, the antibodies are rarely neutralizing and the autoantibody production never reaches the levels observed in APECED patients (Meager et al., 2003; Rice et al., 2013). Particularly in mouse models, many datasets suggest that type I IFNs contribute to T1D (Carrero et al., 2013; Downes et al., 2010; Li et al., 2008). There has been observed a unique correlation between antibody-mediated neutralization of IFN-α and failure to develop T1D. This provides support for animal studies arguing that targeting type I IFNs could be effective in T1D (Kisand et al., 2008; Meyer et al., 2016).

It is known that CMC correlates with circulating autoantibodies against Th17-related cytokines IL-17F and IL-22 (Kisand et al., 2010; Puel et al., 2010). The potential pathogenicity of the IL-22-neutralizing autoantibodies, isolated from APECED patients, has been confirmed in a mouse model of oropharyngeal candidiasis (Bichele et al., 2018). The secretion of respective cytokines is severely impaired in the circulating and skin-residing CD4+ T cells (Kisand et al., 2010; Ng et al., 2010; Puel et al., 2010). Whether the susceptibility for candidiasis is primarily caused by the impaired production of IL-22 and IL-17A or by the neutralizing autoantibodies to Th17 cytokines is not known. Some follow-up studies of the siblings of APECED patients have indicated that autoantibodies to IL-17F and IL-22 can persist to at least 5 years of age without causing any signs of CMC (Sarkadi et al., 2014; Wolff et al., 2013). In addition, patients without current autoantibodies also present CMC, therefore it is hypothesized that in APECED patients' other immune mechanisms may contribute to CMC. APECED patients have an altered immune response to *C. albicans* including dysregulation of IL-23p19 production in monocytes, which might contribute to the selective susceptibility to CMC (Bruserud et al., 2017). It is reported that APECED patients or controls do not have auto-antibodies against IL-23 (Bruserud et al., 2017; Kärner et al., 2016).

Kärner et al. demonstrated that immunoglobulin IgGs but not IgAs in APECED sera are responsible for neutralizing IFN-ω, IFN-α2a, IL-17A, and IL-22. Their dominant subclasses proved to be IgG1 and, surprisingly also IgG4, but without IgE. This is implicating that possibly Treg responses and/or epithelia in their initiation are in these AIRE-deficiency states (Kärner et al., 2013).

3. SUMMARY OF THE LITERATURE REVIEW

In recent years, the relationships of cytokines with different autoimmune diseases have frequently been studied to discover better approaches for targeting pathological causes and to find the best diagnostic methods. Monogenic diseases are unique models for distinguishing various immune mechanisms, including the role of cytokines in immune protection and pathology.

Type I IFNs are known for their crucial role in protection against viral infections. Nevertheless, their excessive production potentiates different pathologies, including vascular abnormalities and autoimmunity. It is known that *STAT1* GOF disease causing variants lead to defective Th17 cell development and chronic mucocutaneous candidiasis, but they also frequently lead to autoimmunity. Although this effect is thought to be the result of *STAT1* GOF cells' inhibition of STAT3 function, the precise molecular mechanism behind the signaling pathway in these patients is not known. In addition, the cause is yet to be discovered for the frequent autoimmune manifestations in *STAT1* GOF patients.

IL-27 has a dual function – depending on the context, it can be pro- or anti-inflammatory. IL-27 is crucial for T cell differentiation and survival by signaling through the STAT pathway. GWAS studies have associated a SNP rs4788084[T] close to the *IL27* gene with protection against T1D. However, it is not known how the variant mediates this effect. Likewise, it is not clear if IL-27 is protective or pathogenic in T1D.

APECED, caused by disease-causing variants in the *AIRE* gene, is an efficient model for studying IL-22 paucity. Th17-related cytokines synergistically up-regulate protective AMPs, but IL-22 has distinct functions in mucosal barrier surveillance. Earlier publications have hinted that APECED patients suffer from chronic mucocutaneous candidiasis due to neutralizing autoantibodies against IL-22 and IL-17F and due to the absence of Th22 cells. Most knowledge about the functions of IL-22 has been derived from mouse and *in vitro* experiments. It is still not clear what events are occurring in the human oral mucosa in the model of IL-22 paucity.

The unique features of monogenic diseases with immune dysregulation enable us to study the role of cytokines in the human immune system.

4. AIMS OF THE STUDY

The general purpose of this thesis was to dissect the role of several STAT1 and STAT3 signaling cytokines in the setting of monogenic diseases: *STAT1* GOF and APECED, and genetic variation of IL-27.

The more specific aims were the following:

- to clarify the consequences of *STAT1* GOF disease causing variants on lymphocyte responsiveness to IFN-α and IL-21;
- to find out if the bioactivity of IL-27 depends on the genetic variation associated with protection against T1D;
- to test the hypothesis that IL-22 paucity in APECED patients can cause alterations in their oral mucosa.

5. MATERIAL AND METHODS

5.1. Study subjects

The use of human samples (Table 1) was approved by local ethics committees (Slovenia: National Medical Ethics Committee number 22/09/09 and 28/02/13; Estonia: Research Ethics Committee of the University of Tartu, 235/M-23). Informed consent was obtained from all participants or parents of participating children.

Table 1. Overview of the study groups.

Study number	Study subjects	Experiments
Study I	5 *STAT1* GOF patients (1 male, 4 females) (age 5–43 years) and age-matched controls	• IFN measurement with digital-ELISA assay (4 patients [8 samples, including 5 follow-up samples from 1 patient] and 35 controls) • Gene expression by quantitative reverse transcription polymerase chain reaction (qRT-PCR) (fresh blood 4 patients and 10 controls, peripheral blood mononuclear cells (PBMCs) 4 patients and 7 controls) • Flow cytometry (whole blood 2 patients and 3 controls, PBMCs 4 patients and 7 controls, PBMCs *STAT1* GOF variant validation 1 patient and 2 controls) • Chromatin immunoprecipitation (ChIP) (4 patients and 6 controls) • ChIP coupled with high-throughput sequencing (ChIP-seq) library preparation and sequencing (4 patients and 6 controls) • Statistical analysis and annotation of ChIP-seq data (4 patients and 6 controls)
Study II	4 controls (age 25–46 years)	• PBMC isolation • PBMC stimulation with wild-type IL-27A, mutant IL-27A and EBI3 • RNA extraction and cDNA synthesis • Primer design • Gene expression by qRT-PCR
Study III	13 patients with APECED (9 males, 4 females) from Slovenia and Estonia (age 4–55 years) and age-matched controls	• Flow cytometry (8 patients and 8 controls) • Autoantibodies from plasma (13 patients and 7 controls) and saliva (10 patients and 10 controls) with luciferase based immunoprecipitation system (LIPS) • Cytokines from saliva (10 patients and 8 controls, with exceptions) and plasma (8 patients and 9 controls, with exceptions) • *Candida* concentration measurement (6 APECED patients, 6 control samples and as a positive control 1 S*TAT1* GOF and 1 *STAT3* loss-of-function (LOF)) • Gene expression from buccal biopsy (4 patients and 4 controls) • Sequencing, processing, and statistical analysis of bacterial 16S rDNA sequences (6 patients and 6 controls)

5.2. Patient characteristics

Table 2. The characteristics of *STAT1* GOF patients. The laboratory and clinical parameters of two patients (P2 and P3) have been published before (Nielsen et al., 2015; Smyth et al., 2018).

Code	Dominant variants of *STAT1* gene (NM_007315.4)	*STAT1* domain	Sex	Sample collection age	Infection phenotype	Autoimmunity and other complications	Laboratory findings	Medication
P1	NM_007315.4(STAT1): c.1169T>C; p.Met390Thr	DBD	F	5, 9, 10 and 14	CMC from infancy, bacterial infections	enteropathy, growth retardation, hypothyroidism	IgG4 deficiency, low memory B cell, Th17 cell deficiency	azoles
P2	NM_007315.4(STAT1): c.800C>T; p.Ala267Val	CCD	F	43	CMC, psoriasiform hyperkeratosis and dermatophytosis, staphylococcal abscesses (Nielsen et al., 2015)	–	normal Ig, normal IgG2, Th17 cell deficiency (Nielsen et al., 2015)	terbinafine, prophylactic trimethoprim-sulfamethoxazole, itraconazole (Nielsen et al., 2015)
P3	NM_007315.4(STAT1): c.848T>C; p.Leu283Ser	CCD	F	7, 8 and 9	CMC, bronchiectasis from recurrent respiratory tract infections, recurrent oral ulceration (Smyth et al., 2018)	combined immunodeficiency, hypothyroidism, episodic diarrhea, iron deficiency anemia, aortic calcification (Smyth et al., 2018)	T cell lymphopenia, low memory B cells, normal Ig	thyroxine, ferrous, fumarate, sulfamethoxazole-trimethoprim, miconazole, ruxolitinib, IVIg (Smyth et al., 2018)
P4	NM_007315.4(STAT1): c.1166T>C; p.Val389Ala	DBD	F	9	recalcitrant CMC from infancy, frequent bacterial infections	–	Th17 cell deficiency	azoles
P5	NM_007315.4(STAT1): c.821G>A; p.Arg274Gln	CCD	M	14	CMC, previous non tuberculous mycobacterial neck abscess	positive antinuclear antibodies (speckled appearance)	defective proliferation to *Candida* antigen, normal Ig, high IgE	–

CCD, coiled-coil domain; DBD, DNA-binding domain.

Table 3. The characteristics of APECED patients. None of the patients or controls reported symptoms of sicca syndrome nor had difficulties in saliva collection, nor received immunosuppressive treatment. Patients did not get antifungal treatment, except PT8 (Lamisil, Mikonazol gel) (not measured: PT6, PT12, PT13). Autoantibodies against IFN-α, IL-17A/F, and IL-22 from plasma were detected in all patients, except anti-IL-17A in PT7 and anti-IL-17A/F in PT12. Autoantibodies from saliva were tested in all patients, except PT6, PT12 and PT13. Flow cytometry was done in all patients, except PT1–PT4 and PT6. Expression analysis was conducted on patients PT1–PT4. Microbiota analysis was conducted on patients PT5, PT7–PT11. *Candida* was measured from saliva, the patient samples which exceeded control mean ± 3 standard deviations of the mean were marked in bold. Controls are age-gender adjusted and recruited at the same time with the patients.

Case	**Autosomal recessive variants of *AIRE* gene (NM_000383.3)**	**Sample collection age**	**Sex**	**Clinical phenotype**	***Candida* infection in mouth at the sampling**	**Mouth swab / *Candida* cells per ml of saliva**
PT1	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.47C>T; p.(Thr16Met)	21	F	CMC, HP, MA, EH	no changes visible on mucous membranes	*Candida* positive
PT2	NM_000383.3(AIRE):c.769C>T, p.(Arg257*) / NM_000383.3(AIRE):c.47C>T; p.(Thr16Met)	16	M	CMC, AD, HP, HT, MA, EH, AL	no changes visible on mucous membranes	*Candida* neg
PT3	NM_000383.3(AIRE):c.21_43dup; p.(Arg15Hisfs*5) / NM_000383.3(AIRE):c.21_43dup; p.(Arg15Hisfs*5)	15	M	CMC	no changes visible on mucous membranes	*Candida* neg
PT4	NM_000383.3(AIRE):c.1067_1071dup; p.(Gln358 Glyfs*22) / NM_000383.3(AIRE):c.1067_1071dup; p.(Gln358 Glyfs*22)	23	M	CMC, HP, GI, HT, MA, EH	numerous candidal plaques in the mouth, redness of the mucosa	*Candida* positive
PT5	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	26	M	CMC, AD, HP, HT, K	candidal plaques on the tongue	*Candida* positive / **5281 cells**
PT6	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.47C>T; p.(Thr16Met)	23	M	CMC, AD, GI, GHD, HT, PF, K, EH, ND, DD		

Case	Autosomal recessive variants of *AIRE* gene (NM_000383.3)	Sample collection age	Sex	Clinical phenotype	*Candida* infection in mouth at the sampling	Mouth swab / *Candida* cells per ml of saliva
PT7	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	33	M	CMC, AD, HP, MA, V, AL, PV, E, AC, HC, HTE, DY	no changes visible on mucous membranes	*Candida* positive / **501 cells**
PT8	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	20	M	CMC, AD, GI, AIH, AL, STD, VA, SS, DP, SD, OB, GLI, IR, AN, SD/AIH	the plaques of trush visible on the tongue, buccal mucosa, and palate	*Candida* positive / 110 cells
PT9	NM_000383.3(AIRE):c.21_43dup; p.(Arg15Hisfs*5) / NM_000383.3(AIRE):c.21_43dup; p.(Arg15Hisfs*5)	8	M	CMC, HP	angular cheilitis	minimal *Candida* / 72 cells
PT10	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	4	F	CMC, HP, EH	normal mucous membranes	*Candida* positive / 54 cells
PT11	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.653-6_-4del; (?)	15	F	CMC, AD, HP, GI, GHD, K	no changes visible on mucous membranes	*Candida* neg / 36 cells
PT12	NM_000383.3(AIRE):c.274C>T; p.(Arg92Trp) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	55	F	HP, AD		
PT13	NM_000383.3(AIRE):c.769C>T; p.(Arg257*) / NM_000383.3(AIRE):c.769C>T; p.(Arg257*)	12	M	CMC, AD, HP, ND		

CMC, candidiasis; AD, Addison's disease; HP, hypoparathyroidism; GI, gonadal insufficiency; T1D, type 1 diabetes mellitus; GHD, growth hormone deficiency; HT, hypothyroidism; PA, pernicious anemia; AIH, autoimmune hepatitis; MA, malabsorption; GID, gastrointestinal dysfunction; D, diarrhea; O, obstipation; TIN, tubulointerstitial nephritis/kidney disorders related to APECED; K, keratitis/keratopathy; EH, enamel hypoplasia; VIT, vitiligo; AL, alopecia; ND, nail dystrophy; AS, asplenia; PV, pityriasis versicolor; E, epilepsy; AC, allergy to carbamazepine; HC, hypercholesterolemia; HTE, hypertension; DY, dysphagia; STD, staphylodermia; VA, varicella; SS, short stature; DP, delayed puberty; SD, steroid diabetes; OB, obesity; GLI, glucose intolerance; IR, insulin resistance; AN, acanthosis nigricans; SD/AIH, steroid diabetes during relapse of autoimmune hepatitis; B, bronchiectasis; OS, osteoporosis.

5.3. Interferon measurement with Simoa assay (Study I)

IFN-α concentration was detected from 4 *STAT1* GOF patients (8 samples, including 5 follow-up samples from 1 patient, and 35 controls). IFN-α protein levels were quantified with a digital-ELISA assay (SIMOA, Quanterix) developed as previously described (Rodero et al., 2017) in accordance with the manufacturer's Homebrew Simoa assay kit instructions, utilizing two autoantibodies specific for IFN-α isolated and cloned from two recently described APECED patients (Meyer et al., 2016). Each serum sample was analyzed in duplicate. The limit of detection (0.33 fg/ml) was calculated as the mean value + 3 standard deviations of reactivity from all blank runs.

5.4. Gene expression by qRT-PCR (Study I, II)

In Study I fresh blood samples (4 *STAT1* GOF patients and 10 controls) were collected in TempusTM Blood RNA tube. Total RNA was separated from blood with TempusTM Spin RNA Isolation Reagent Kit (Applied Biosystems) according to the manufacturer's protocol. Isolated PBMCs (4 *STAT1* GOF patients and 7 controls) were stimulated with IFN-α (10^4 U/ml) (Miltenyi Biotec) and IL-21 (25 ng/ml) for 3 hours and 24 hours or left unstimulated. In Study II isolated PBMCs (4 controls, one person was used in two parallel sets) were stimulated with either mutant IL-27A (NM_145659.3:c.356T>C) (150 M/LU), wild-type IL-27A (150 M/LU) and EBI3 (150 M/LU) for 5 hours or left unstimulated. In Study I and II total RNA was extracted from PBMCs with RNeasy Micro Kit with the recommended RNase-free DNase I treatment (Qiagen) according to the manufacturer's protocol. RNA concentrations were measured with NanoDrop (Thermo Scientific, Waltham, MA) and stored at –80 °C. Reverse transcription was performed on 5 μg of total RNA using Superscript III (Invitrogen), 10 mM dNTP Mix (Fermentas), RiboLock RNase inhibitor (Fermentas). Real-time quantitative PCR (qPCR) was done using Applied Biosystems® ViiA™ 7 Real-Time PCR System with 384-Well Block (Life Technologies) and Maxima SYBR Green /ROX qPCR Master Mix (Fermentas). Each sample was run in 3 parallel reactions. The relative gene expression levels were calculated using the comparative Ct method and normalized to the expression of housekeeping gene β-actin. In Study II primers for *STAT1* and *IRF1* were designed to span the area that is covered by the Illumina gene expression probe. Primers used are listed in Paper I, Supplementary Table S5 and Paper III, Supplementary material publications.

5.5. Flow cytometry (Study I, III)

PBMCs from 4 *STAT1* GOF patients and 7 controls (Paper I) and 1 *STAT1* GOF patient and 2 controls (Paper II) were stimulated with IFN-α (10^4 U/ml) (Miltenyi Biotec) and IL-21 (25 ng/ml) (PeproTech) for 30 min, 1 hour, and 3 hours or left unstimulated at 37 °C, fixed with BD Cytofix Fixing buffer (BD Biosciences), permeabilized with Perm Buffer III (BD Biosciences) and stained with respective antibodies according to manufacturers' protocol. Unstimulated whole blood samples from 2 *STAT1* GOF patients and 3 controls (Study I) were fixed with 1x Lysing buffer (BD Biosciences) and stained as above. In Study III 8 APECED patient and 8 control peripheral blood was drawn into heparinized vacutainers, separated into plasma and PBMCs, and stored at –20 °C or liquid nitrogen respectively. PBMCs were stained in flow cytometry buffer (PBS (pH 7.2), 2mM EDTA, 0.5% BSA) for 20 min at room temperature in dark with antibodies. The antibodies were for Study I: AF647 mouse anti-STAT1 (pY701); PE mouse anti-STAT3 (pY705) (all from BD Biosciences); Study III: APC-Cy7 CD56, Brilliant Violet 650 CD3, PE TCR Vd2, Brilliant Violet 510 TCR va7, PerCP-Cy5.5 CD161 (all from Biolegend), FITC TCR Vd1 (Thermo), APC CD1d dextramer (Immudex). All samples were measured with LSRFortessa (BD Biosciences) using FACSDiva version 6 (BD Biosciences) software and analyzed with FCS Express 5 (De Novo Software).

5.6. Chromatin immunoprecipitation (Study I)

ChIP immunoprecipitation (ChIP) assays were performed as previously described (Tripathi et al., 2017) with few modifications. 4 *STAT1* GOF patient and 6 control PBMCs were fixed immediately after PBMC thawing or isolation. Cells were sonicated using the Bioruptor UCD-200 sonicator (Diagenode) to obtain chromatin fragments of 200–400 bp. Fragmented chromatin was immunoprecipitated with 2 µl of anti trimethylation of lysine 4 of histone 3 (H3K4me3) antibody (#07-473, Millipore) and 2 µl of Dynabeads M-280 magnetic beads (Sheep Anti-Mouse IgG, Sheep Anti-Rabbit IgG, Life Technologies) using the SX-8G IP-Star Automated System (Diagenode). ChIP samples were decross-linked for 4 hours at 65 °C. After incubation, the samples were treated with 0.4 mg/ml Proteinase K (Thermo Scientific) and 0.2 mg/ml RNaseA (RNase Cocktail Enzyme Mix, Ambion, Life Technologies). DNA was extracted with the DNA Clean kit & Concentrator TM-5 (Zymo Research) according to the manufacturer's protocol. The ChIP and input DNAs were quantified with Qubit 2.0 Fluorometer (Life Technologies). Libraries were prepared with the Ovation® Ultralow Systems Kit (NuGeneration Limited, England and Wales) according to the manufacturer's protocol. The libraries were quantified by Qubit 2.0 Fluorometer and validated with Agilent 2200 TapeStation analysis (Agilent Technologies, Inc.). Samples were sequenced with HiSeq 2500 (Illumina, San Diego, USA) producing single-end 50 bp sequences. Sequenced data were demultiplexed

with the CASAVA 1.8.2 software (Illumina, San Diego, USA). Data processing steps were carried out in the High Performance Computing Center of the University of Tartu. The quality control was done with FastQC version 0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw reads were trimmed with Trim Galore! version 0.3.3 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to filter out low-quality bases the Phred score > 30 was used. The reads were aligned to the reference genome with bowtie2 version 2.0.2 (Langmead & Salzberg, 2012). Peaks were called with MACS2 version 2.1.0 (https://github.com/taoliu/MACS/). Only the peaks with q-value < 0.01 were considered significant. The analysis of differentially enriched peaks was performed with the R/Bioconductor packages DiffBind (Ross-Innes et al., 2012) and ChIPseeker (Yu et al., 2015) in R statistical software (http://www.r-project.org/). Sequencing data were visualized with the IGV genome browser (Robinson et al., 2011). The Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean et al., 2010) was used with default settings to link differentially enriched ChIP-seq peaks with external annotation sources. The 15-state ChromHMM model of peripheral blood mononuclear cells (PBMCs E062) from the Roadmap Epigenomics Consortium (Kundaje et al., 2015) data repository, was used to determine the specific functionally-defined regions that overlap with differentially enriched ChIP-seq peaks. Publicly available ChIP-seq datasets of transcription factors STAT1, STAT2 and STAT3 binding sites (Paper I, Supplementary Table S2) were used to compare with differentially enriched ChIP-seq peaks together with 5000 bp upstream and downstream of the peak. Based on quality score the STAT datasets were trimmed to top 75%. The differentially enriched ChIP-seq peaks which were closer than 2000 bp were merged (59 sites instead of 65) and used in the analysis. To compare STAT1 binding sites between differentially enriched regions and H3K4me3 regions a random subset of 59 regions was selected from all the peaks and the selection was repeated 10 000 times. The p-value was calculated by dividing the number of trials that resulted in an equal number or more overlaps than with the differentially enriched regions with the number of random selections. The g:Profiler web server (Raudvere et al., 2019) was used to annotate the genes associated with H3K4me3 sites. The datasets were deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE120580.

5.7. Autoantibodies from plasma and saliva with LIPS (Study III)

LIPS assay was conducted on plasma samples (13 APECED patients and 7 controls) and saliva samples (10 APECED patients and 10 controls). Peripheral blood was drawn into heparinized vacutainers, separated into plasma and PBMCs, and stored at –20 °C or liquid nitrogen, respectively. The saliva samples were provided using the passive-drool method, in which participants allow saliva to pool

in the mouth and then drool it into a tube. Samples were stored immediately at –80 °C. LIPS assay was done as previously described (Kärner et al., 2013). Briefly, IL-22, IL-17F, IL-17A, and IFN-α coding sequences were cloned into a modified pPK-CMV-F4 fusion vector (PromoCell GmbH, Germany) where Firefly luciferase was substituted with NanoLuc luciferase (Promega, USA). Cloned constructs were transfected into HEK293 cells and secreted luciferase fusion proteins were collected after 48 hours. IgG from saliva and plasma samples was captured onto Protein G Agarose beads (Exalpha Biologicals, USA) at room temperature for 1 hour (Merck Millipore, Germany). Next, $1x10^6$ luminescence units (LU) of antigen was added. After 1 hour the unbound antigen was washed away with a vacuum system and Nano-Glo® Luciferase Assay Reagent (Promega, USA) was added. Luminescence intensity was measured with VICTOR X Multilabel Plate Reader (PerkinElmer Life Sciences, USA). The results were indicated as LU representing the fold over the mean of the control samples.

5.8. Cytokines from plasma and saliva (Study III)

Cytokine quantification was conducted on plasma samples (8 Slovenian APECED patients and 9 controls, except IL-1β, IL-2, IL-5, IL-6, IL-10, TNF-α, GM-CSF, IFN-γ, which were measured from 7 patient and 8 control) and saliva samples (10 Slovenian patients and 8 controls, with the exception for IL-1β, IL-2, IL-5, IL-6, IL-10, TNF-α, GM-CSF, IFN-γ, which were measured with 6 patient samples and 5–6 control samples). Peripheral blood was drawn into heparinized vacutainers, separated into plasma, and stored at –20 °C. The saliva samples were provided using the passive-drool method, in which participants allow saliva to pool in the mouth and then drool it into a tube. Samples were stored immediately at –80 °C. Cytokine levels were measured by the xMAP Technology on Luminex 200 (Luminex Corp., Austin, TX) with Human Magnetic Luminex Performance Assay Kits (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's protocol. The cytokines studied can be found in Table 4. In addition, IL-1α from plasma sample, IL-2, IL-5, IL-17A, IL-17F, and IL-22 fell below the detection limit in both plasma and saliva samples, therefore they were excluded from the results.

Table 4. Cytokines detected from saliva and plasma. IL-2, IL-5, IL-17F, IL-17A, and IL-22 fell below the detection limit in both plasma and saliva samples, therefore they were excluded from the results. In addition, in plasma samples the measurements of IL-1α were below the detection limit, therefore they were excluded from the results. The lower limit of detection was determined according to the manufacturer.

Cytokine	LOD	Sample	Patient n/ Control n	Patient mean (pg/ml) ± SD	Control mean (pg/ml) ± SD
IL-36β	0.49	Saliva	10 / 8	350.8 ± 232.6	632.9 ± 749.9
		Plasma	8 / 9	3.54 ± 6.87	4.73 ± 10.24
IL-7	0.80	Saliva	10 / 8	9.26 ± 3.70	9.63 ± 8.54
		Plasma	8 / 9	20.36 ±10.29	19.25 ± 14.89
TNF-α	0.81	Saliva	6 / 6	123.0 ± 74.79	20.85 ± 9.17
		Plasma	7 / 8	10.39 ± 3.97	8.02 ± 2.73
IL-1β	0.34	Saliva	6 / 6	452.7 ± 405.0	118.9 ± 120.2
		Plasma	7 / 8	1.13 ± 0.40	1.01 ± 0.57
IL-6	0.93	Saliva	6 / 6	16.50 ± 6.82	12.77 ± 14.79
		Plasma	7 / 8	1.70 ± 0.37	1.74 ± 1.54
IL-10	0.46	Saliva	6 / 5	2.49 ± 2.90	1.65 ± 1.78
		Plasma	7 / 8	5.47 ± 11.40	0.67 ± 0.55
GM-CSF	0.39	Saliva	6 / 5	4.04 ± 0.83	1.47 ± 0.37
		Plasma	7 / 8	0.57 ± 0.44	0.49 ± 0.67
IFN-γ	0.31	Saliva	6 / 5	1.10 ± 0.69	0.04 ± 0.08
		Plasma	7 / 8	0.54 ± 1.25	0.07 ± 0.06
CXCL10/IP10	3.65	Saliva	10 / 8	398.7 ± 345.4	168.9 ± 94.07
		Plasma	8 / 9	133.2 ± 73.92	75.24 ± 23.16
LCN2	41.15	Saliva	10 / 8	99355 ± 26968	119377 ± 27764
		Plasma	8 / 9	33938 ± 22667	32814 ± 18470
G-CSF	5.83	Saliva	10 / 8	72.96 ± 44.06	96.21 ± 37.96
		Plasma	8 / 9	22.01 ± 11.32	20.32 ± 8.52
IL-1α/IL-1F1	2.88	Saliva	10 / 8	349.4 ± 196.0	572.7 ± 540.8
		Plasma	-	–	–
IL-1ra/IL-1F3	6.52	Saliva	10 / 8	64908 ± 20442	73890 ± 34548
		Plasma	8 / 9	1123 ± 856.8	1645 ± 548.3
CXCL8/IL-8	3.77	Saliva	10 / 8	3255 ± 3023	1727 ± 1211
		Plasma	8 / 9	14.31 ± 7.86	12.53 ± 16.10

LOD, limit of detection; SD, standard deviation.

5.9. Gene expression from buccal biopsy (Study III)

Buccal biopsy samples were collected from 4 APECED patients and 4 age-matched controls. Buccal biopsies were taken after local anesthesia with a surgical scalpel under aseptic conditions. However, 2 control samples did not pass the RNA quality control after RNA isolation, and therefore, the gene expression analysis was conducted on 4 APECED patients and 2 control buccal biopsy samples. The samples were homogenized in TRIzol (Invitrogen) and RNA was extracted with the miRNeasy Mini Kit combined with the RNase-free DNase I treatment (both from Qiagen). The concentration and quality were assessed with an Agilent RNA 6000 Nano Kit with Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). cRNA was prepared from 300 ng of total RNA using an Illumina TotalPrep RNA Amplification Kit (Ambion Inc., TX, USA) according to the manufacturer's protocol. A genome-wide gene expression analysis was carried out using HumanHT-12 v4 Expression BeadChip (Illumina Inc., CA, USA) and the signals were scanned using a Beadscan (Illumina Inc.). Array data were analyzed using GenomeStudio software (Illumina, San Diego, CA, USA). Rank invariant normalization was done. Genes with the FDR adjusted p-value < 0.05 and a fold change >1.5 were considered as differentially expressed. A positive diffScore represented upregulation, while a negative diffScore represented downregulation. The p-value for an observed expression difference between two analyzed groups was < 0.05 for genes with diffScores of lower than –13 and higher than +13 (p-value < 0.01 is –20 > diffScore > 20; p-value < 0.001 is –33 > diffScore > 33). Negative average signal value was excluded from the list. Annotation analysis was conducted with g:Profiler software (Raudvere et al., 2019) and Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/). Analyzed data set included the upregulated and downregulated gene list with expression fold change. The core analysis was carried out with reference set Ingenuity Knowledge Base (Genes only), by default settings. Diseases and functions analysis was conducted, which predicted effected biology based on gene expression. P-value was calculated based on a Right-Tailed Fisher's Exact Test. Z-score indicated predictions about upstream or downstream processes. Network analysis generated the scores (p-score is -log10 (p-value)) based on the fit of the set of supplied genes and a list of biological functions stored in the Ingenuity Knowledge Base. Upstream regulator analysis was used to predict the upstream transcriptional regulators from the dataset based on the literature and compiled in the Ingenuity Knowledge Base, which calculated the causal networks. Participating regulators were molecules through which the upstream regulator molecule controls the expression of target molecules.

5.10. *Candida* detection from saliva (Study III)

The concentration of *Candida* was measured in 6 APECED patients, 6 control samples and as a positive control in two patients with different monogenic diseases with CMC – *STAT1* GOF and *STAT3* LOF. None of the 6 controls had symptoms of *Candida* infection. The saliva samples were provided using the passive-drool method, in which participants allow saliva to pool in the mouth and then drool it into a tube. Samples were stored immediately at –80 °C. DNA of *C. albicans* was extracted from saliva samples and cultured laboratory strain SC5314 (ATCC, VA, US) using the DNeasy PowerSoil Kit (QIAGEN) according to the manufacturer's protocol. Samples were homogenized with Precellys 24 Homogenizer (Bertin Instruments) with program 6500 rpm, 2-minute homogenization with a 1-minute break. Real-time qPCR was performed using Applied Biosystems® ViiA™ 7 Real-Time PCR System with 384-Well Block (Life Technologies) and Maxima SYBR Green /ROX qPCR Master Mix (Fermentas). A primer pair of highly conserved rDNA-coding region 28S of *Candida* was used (forward *CGGCGAGTGAAGCGGCTAA*, reverse *ATTCCCAAACAACTCGACTC*) (Zhang et al., 2016). The concentration of *Candida* cells was calculated based on the calibration curve constructed according to the Ct values of serially diluted *C. albicans* probes (starting from 2E7 colony stimulating factor (CFU)/ml). The normal range of *Candida* concentration was calculated based on values of control subjects (mean + 3 standard deviations).

5.11. Sequencing of bacterial 16S rDNA (Study III)

The experiment was conducted on 6 APECED patient and 6 control samples. The saliva samples were provided using the passive-drool method, in which participants allow saliva to pool in the mouth and then drool it into a tube. Samples were stored immediately at –80°C. DNA from saliva samples was extracted using the MolYsis™ Complete 5 Kit according to the manufacturer's protocol. The bacterial 16S rDNA variable region V1-V2 amplicon libraries were prepared in a dual indexing approach using the 27F-338R primer combination. Sequencing was performed on an Illumina MiSeq using 2x300 cycles and MiSeq Reagent Kit v3 (Caporaso et al., 2012). Data were processed using the DADA2 workflow (https://benjjneb.github.io/dada2/; v1.10) and subsequently analyzed in R using the vegan and phyloseq packages. Taxonomic annotation was conducted using a Bayesian classifier and RDP training set 16. 20k sequences were randomly sampled per each sample for a normalized read count. The analysis was done on the amplicon sequence variant (ASV), species, and genus level. For differentially abundant taxonomic groups all tests were conducted using the Wilcoxon rank-sum test using only taxa with median abundance > 100 sequence counts (0.5% rel. abundance) and prevalence > 0.3 (present in at least 4 samples). Alpha diversity (within sample diversity) analysis was performed with Wilcoxon rank-sum tests, diversity indices were used (Observed – Observed taxa with

abundance > 0, Shannon – Shannon Diversity Index, Chao1 – Chao1 Estimator) to estimate observed and unobserved species count. Differences in beta diversity (Whole Community Composition / inter-sample diversity) were assessed using Bray-Curtis similarity and permutational multivariate analysis of variance (*adonis*() function of the *vegan* package in R software). The sequences are deposited in the NCBI Sequence Read Archive Database (https://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA601650.

5.12. Statistical analysis

Statistical analysis was performed using GraphPad Software (San Diego, CA) and R statistical software (https://www.r-project.org/). Unpaired two-tailed t-tests, Wilcoxon rank-sum tests or permutational ANOVA tests were used to assess the differences between studied groups.

6. RESULTS AND DISCUSSION

6.1. Cytokines and STAT1 (Study I, II)

6.1.1. Hyperphosphorylation of STAT1 in *STAT1* GOF patients (Study I)

STAT1 signaling pathway is activated mostly through IFNs but also by other cytokines, such as IL-21 and IL-27 (Spolski & Leonard, 2008; Thyrell et al., 2007). GOF variants in the *STAT1* gene lead to hyperphosphorylation and delayed dephosphorylation of STAT1 during cytokine responses (Liu et al., 2011; Meesilpavikkai et al., 2017; Smeekens et al., 2011; van de Veerdonk et al., 2011; Yamazaki et al., 2014). This effect was replicated in our study – after IFN-α and IL-21 stimulation, the patients' cells demonstrated a significantly higher level of p-STAT1 induction in comparison to age-matched control PBMCs (Fig. 8A and B).

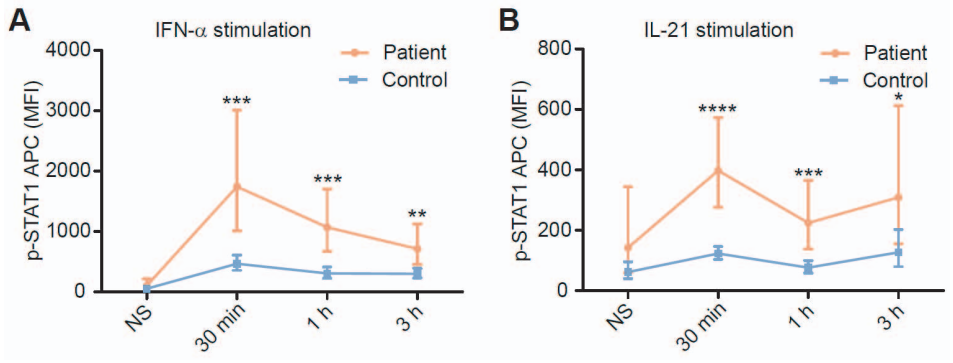

Figure 8. STAT1 phosphorylation levels in *STAT1* GOF patients. The phosphorylation of STAT1 was measured in PBMCs with flow cytometry, and cells were left unstimulated (NS) or stimulated with IFN-α (A) or IL-21 (B). Results are shown as geometric mean fluorescence intensity (MFI) ×÷ geometric standard deviation from 4 patients and 7 controls. Data were pooled from four independent experiments with one patient and 1–2 controls in each. Statistical significance was assessed by the unpaired two-sample t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

6.1.2. Case study of a *STAT1* GOF patient (Study I)

To confirm the nature of the novel variant (c848T>C, [p.Leu283Ser]) in the *STAT1* gene, peripheral blood mononuclear cells (PBMC) from the patient and two controls were stimulated with IFN-α (10^4 U/ml) (Miltenyi Biotec) for 30 min, 1 hour and 3 hours. Stimulated cells were stained with anti-phospho-STAT1 after fixing and permeabilization, as recommended by the manufacturer. Flow cytometry results confirmed the gain of function in the patient's sample (Fig. 9).

STAT1 GOF disease causing variants were first linked to autosomal dominantly inherited CMC in 2011 (Liu et al., 2011; van de Veerdonk et al., 2011). Since then, the phenotype of patients with GOF variants in STAT1 has broadened considerably and includes susceptibility to a wide array of infections, auto-immunity, aneurysms (cerebral and extra-cerebral), and malignancy (Toubiana et al., 2016). Aortic calcification has been reported only once before with this condition (Uzel et al., 2013). Uzel et al. describe a patient with calcification of the aorta, coeliac artery, and superior mesenteric artery in their case series of patients with *STAT1* GOF disease. Our case study reported an aortic calcification in the patient with combined immunodeficiency due to a novel *STAT1* GOF variant (c848T>C [p.Leu283Ser]) (Smyth et al., 2018).

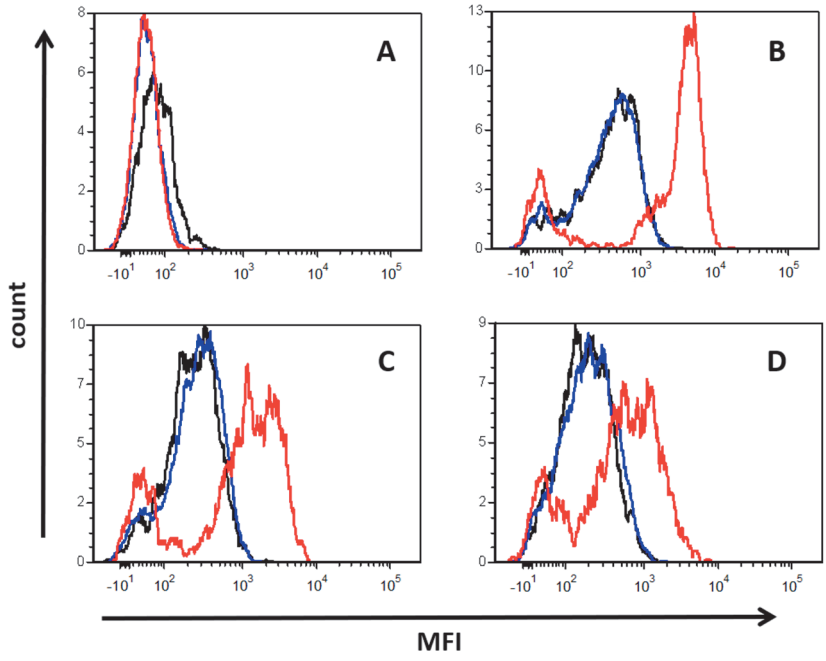

Figure 9. The flow cytometry analysis to confirm the pathogenic variant of *STAT1* GOF cells. The PBMCs were collected from the patient (red) and two controls (blue and black). The MFI level (x-axis) of p-STAT1 was measured at baseline (A), 30 minutes (B), 1 hour (C), and 3 hours (D) after stimulation with IFN-α.

6.1.3. Interferon signature in *STAT1* GOF patients (Study I)

In *STAT1* GOF patients, circulating blood cells revealed the IFN signature – the upregulation of ISGs. To define the consequences of increased responsiveness to IFNs *in vivo*, we measured the serum levels of IFN-α. For the experiment, we applied an ultrasensitive digital ELISA method (Rodero et al., 2017). IFN-α was detectable in most, but not all, of the patients with an available serum sample, but

at low concentrations (median=2.9 fg/ml) (Fig. 10A) in comparison to patients with interferonopathies or SLE who were tested with the same assay (Rodero et al., 2017). In one of the patients, the follow up of IFN-α serum levels showed substantial fluctuations of IFN-α levels, including in samples drawn only 1 week apart (Fig. 10B). When taking the samples, the patient was declared free of any other infections besides recalcitrant CMC. A persistent increase in STAT1 phosphorylation *in vivo* was not observed in patients with low to moderate IFN-α levels. This was shown with blood samples, fixed within 15 minutes after blood sampling, revealing similar p-STAT1 levels in patients and controls (Fig. 10C).

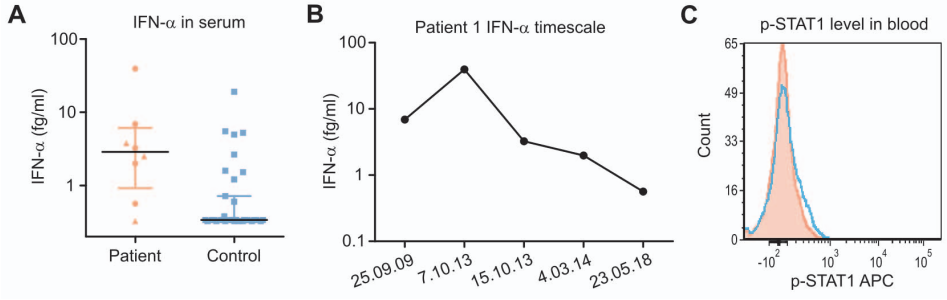

Figure 10. IFN-α levels detected in serum and blood. IFN-α concentration from serum (A) was measured with digital ELISA. Results are shown as median + interquartile range, with n=8 patient samples (n=5 were from the same patient but at different time points, as shown in panel B, indicated as circles) and n=35 controls. The data were pooled from 2 different experiments: 1) 2 patients + 25 controls, 2) 6 patients + 10 controls. A representative result shows the p-STAT1 level (C) in one of two patients compared to one of three controls tested in parallel out of four experiments. Whole blood samples were fixed within 15 minutes after blood sampling, and the cells were stained with anti-p-STAT1. Orange – CMC patient, blue – control.

Nevertheless, the IFN signature was evident in patients' blood cells. Each patient's blood was drawn into Tempus tubes in parallel with controls to immediately preserve the *in vivo* status of mRNA expression (Fig. 11A). Elevated levels of ISGs were seen even if IFN-α concentration was below the detection limit of the assay (0.33 fg/ml) (P2). Only the relatively IFN-γ-specific class II major histocompatibility complex transactivator (CIITA) was not differentially upregulated in *STAT1* GOF patients from the 12 tested ISGs. Intriguingly, the patient who had been treated with the JAK1/2 inhibitor ruxolitinib (P3) for 11 months had dampened ISG expression (Fig. 11B).

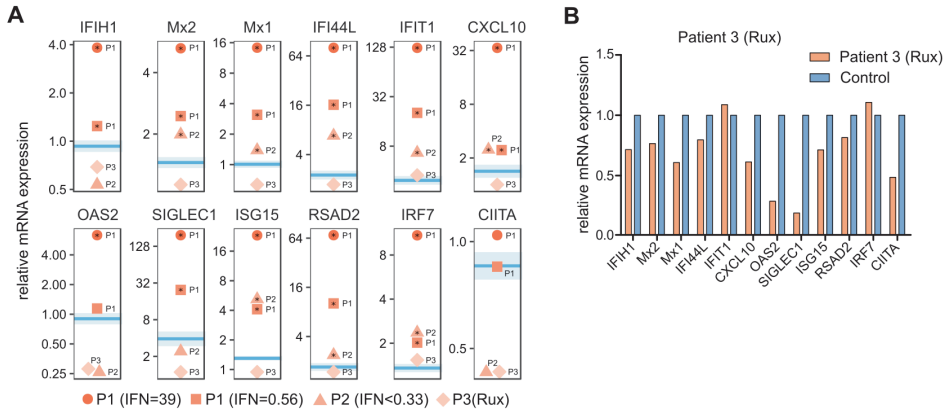

Figure 11. Gene expression of the ISGs. Whole blood samples were collected using a TempusTM Blood RNA tube (3 different patients [4 samples] and 10 controls). The relative gene expression levels were calculated using the ΔΔCt method and normalized to the expression of the housekeeping gene β-actin. In panel A, the data were normalized to the mean of 10 controls who had IFN-α protein levels below 0.33 fg/ml. The horizontal blue line represents the geometric mean of the expression values (fold changes) of the control sample group (n=10). The transparent blue area shows the standard error of the geometric mean. Statistical significance was assessed with the one-sample t-test using Graphpad Prism software, * $p < 0.01$. Panel B was zoomed in to panel A to show the relative gene expression of one patient treated with ruxolitinib (P3) in comparison to the mean of 10 controls.

Taken together, *STAT1* GOF patients exhibited a clear interferon signature. Furthermore, the IFN signature reflected exaggerated responses to relatively moderate levels of IFN-α.

6.1.4. Differential expression of ISGs is epigenetically determined (Study I)

STAT molecules are known to cause chromatin modifications together with other transcription factors in determining immune cell differentiation (Vahedi et al., 2012; Wei et al., 2010). Therefore, we wanted to find out if the dephosphorylation defect of *STAT1* GOF variant could lead to epigenetic changes. We performed ChIP and ChIP-seq analysis of the active chromatin histone mark, the H3K4me3 in PBMCs, from 4 *STAT1* GOF patients and 6 controls. The comparison of binding sites revealed 11.6% (3193) patient-specific peaks and 9.7% (2672) control-specific peaks, and most of the peaks were shared by the patient and control groups in the common group, 78.7% (21609) (Paper I, Supplementary Fig. S2A-B). For the overlapping peaks in the common group, 1/3 of the peaks were associated with genes located within 5 kb upstream of the transcription start site (TSS). The distribution of patient- and control-specific H3K4me3 binding sites was similar – the majority of the regions were located far away from the TSS (5 to 500 kb upstream or downstream), which implies to a role for enhancers in target regulation.

Gene ontology term analysis (Paper I, Supplementary Fig. S2C) revealed that H3K4me3 binding sites of the overlapping common peak set were involved in antigen processing, the type I IFN pathway, and different protein methylation events. We noted that the gene ontology terms of the patient- and control-specific peak sets were similar, and the majority of the peaks were related to lymphocyte activation and differentiation.

To find differentially enriched regions, we analyzed the differential enrichment of H3K4me3. We found 65 sites that were differentially enriched between the patient and control groups (Paper I, Supplementary Table S1). Most of the sites could be associated with at least one gene (Fig 12A). With the help of the Roadmap Epigenomics Consortium (Kundaje et al., 2015) data from the functional genomic states of PBMCs (E062) were assigned to these regions (Fig. 12B). About 40% of the regions covered the active TSS and the flanking active TSS area in the immediate neighborhood of the TSS. In addition, about 20% of the peaks were located in the enhancer regions (Fig. 12B). As an example, Figure 12C shows elevated enrichment of the permissive chromatin mark in a much broader region in patients versus controls, depicting the sequencing read densities around the STAT1 gene TSS. Also, Figure 12C shows the location of the differentially enriched regions relative to the chromatin states defined by the ChromHMM model from PBMCs. Specifically, the differentially enriched H3K4me3 regions overlapped with the flanking active TSS (TssAFlnk) chromatin state, which characterized the actively transcribed genes.

To find the deeper biological meaning behind the differentially enriched peaks, we conducted an ontology analysis. Importantly, gene ontology analysis revealed that increased H3K4me3 binding occurred in patients in the genes that are involved in IFN-γ signaling, response to a virus, and the type I IFN pathway (Table 5). This implied that the IFN signaling pathway in patients was differentially regulated compared to controls.

Next, we wanted to compare the distribution of differentially enriched regions with STAT factors binding sites. To analyze the occurrence of STAT binding sites within or near the differentially enriched H3K4me3 regions, we juxtaposed the list of differentially enriched regions with publicly available STAT1, STAT2 and STAT3 ChIP-seq data sets derived from different cell lines (Paper I, Supplementary Table S2). The comparison determined 37 STAT1 binding sites that overlapped with 42% (25/59) of the differentially enriched regions and that were mostly located at the enhancers, active TSS, or transcription sites (Paper I, Supplementary Table S3). Also, the analysis revealed 13 STAT3 binding sites situated on 14% (8/59) of the regions, of which 4 regions overlapped with the STAT1 sites. 11 STAT2 binding sites covered 15% (9/59) of the regions, most of which overlapped with the STAT1 binding sites located in the TSS areas. The presence of STAT binding sites at the *STAT1* gene promoter is illustrated in Figure 12C.

Taken together, the genome-wide profiling of H3K4me3 modifications in *STAT1* GOF patients revealed a significant enrichment of the permissive chromatin

mark near ISGs, which may explain the increased blood cell ISG expression, even during periods of low or undetectable circulating IFN-α levels.

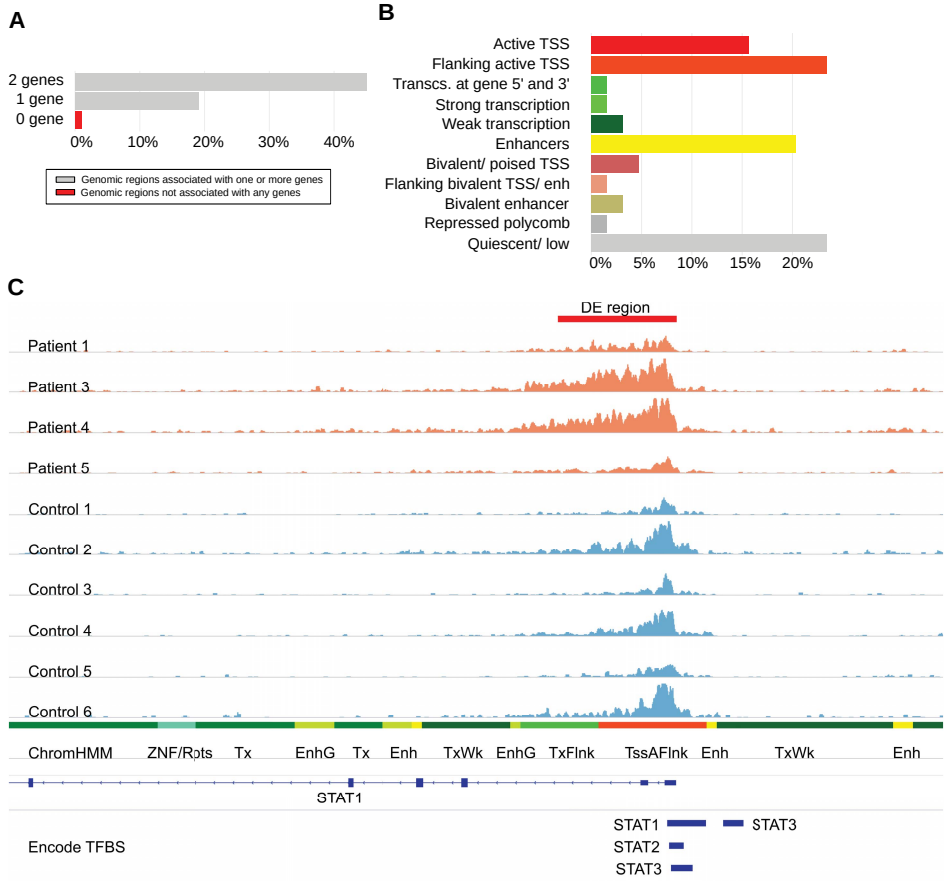

Figure 12. The differentially enriched regions between *STAT1* GOF patients and controls. (A) The bar graph shows the number of associated genes per differentially enriched H3K4me3 region. (B) The bar graph shows the distribution of differentially enriched H3K4me3 regions between various genomic features of the 15-state ChromHMM model for PBMCs. (C) A snapshot of the STAT1 promoter region depicts the H3K4me3 ChIP-seq signal in 4 patients (orange) and 6 control (blue) samples. Altogether, 4 independent ChIP experiments were done with PBMCs from one patient and 1–2 controls in each experiment. Sequencing of the enriched chromatin fragments was performed in one run. The read density signal range of each sample track was set to 0–50. The ENCODE TFBS track displays the binding sites of transcription factors STAT1, STAT2, and STAT3. The ChromHMM track displays the following annotations: ZnF/Rpts, ZNF genes & repeats; Tx, strong transcription; EnhG, genic enhancers; Enh, enhancers; TxWk, weak transcription; TxFlnk, transcription at gene 5' and 3'; TssAFlnk, flanking active TSS.

Table 5. Top 10 biological processes connected with differentially enriched sites. The ontology analysis was conducted on the 65 sites that were differentially enriched between the *STAT1* GOF patient and control groups. The GREAT was used to link differentially enriched ChIP-seq peaks with external annotation sources. The GREAT gene list was annotated with g:Profiler tool for enriched biological terms from Gene Ontology database (GO: BP) and the REACTOME database, $p < 0.05$.

P-value	Term ID	Database	Biological process	Gene list
0.0000206	REAC:877300	REACTOME	Interferon gamma signaling	STAT1, GBP3, GBP1, OASL, GBP5, GBP4, GBP6, HLA-B
0.0000802	REAC:913531	REACTOME	Interferon signaling	STAT1, GBP3, GBP1, IFIT3, RSAD2, OASL, GBP5, GBP4, GBP6, HLA-B
0.0021	GO:0034341	GO: BP	Response to interferon-gamma	STAT1, GBP1, OASL, NLRC5, SNCA, GBP5, GBP6, HLA-B
0.00245	GO:0051607	GO: BP	Defense response to virus	IFIH1, STAT1, GBP3, GBP1, IFIT3, RSAD2, OASL, IFI44L, NLRC5
0.00445	GO:0009615	GO: BP	Response to virus	IFIH1, STAT1, GBP3, GBP1, IFIT3, RSAD2, OASL, IFI44L, IFI44, NLRC5
0.00696	GO:0071357	GO: BP	Cellular response to type I interferon	STAT1, IFIT3, RSAD2, OASL, NLRC5, HLA-B
0.00696	GO:0060337	GO: BP	Type I interferon signaling pathway	STAT1, IFIT3, RSAD2, OASL, NLRC5, HLA-B
0.00916	GO:0034340	GO: BP	Response to type I interferon	STAT1, IFIT3, RSAD2, OASL, NLRC5, HLA-B
0.00956	GO:0071346	GO: BP	Cellular response to interferon-gamma	STAT1, GBP1, OASL, NLRC5, GBP5, GBP6, HLA-B
0.0156	GO:0098542	GO: BP	Defense response to other organism	IFIH1, STAT1, GBP3, GBP1, IFIT3, RSAD2, OASL, IFI44L,NLRC5, TLR1, TLR6, GBP6

GO, gene ontology; BP, biological process.

6.1.5. p-STAT1 and p-STAT3 balance is disturbed (Study I)

The stimulation of *STAT1* GOF cells has an impact on gene expression. It has been shown that cytokine stimulation of *STAT1* GOF cells reveals an exaggerated expression of STAT1-dependent genes (Bloomfield et al., 2018; Martinez-Martinez et al., 2015; Mizoguchi et al., 2014; Wan et al., 2015; Zhang et al., 2017; Zheng et al., 2015) and impaired induction of STAT3-dependent genes (Bloomfield et al., 2018; Zheng et al., 2015). Likewise, in response to IFN-α stimulation, we noticed an upregulated *IFIH1* gene (Fig. 13A) and impaired *TWIST1* expression after IL-21 stimulation of PBMCs (Fig. 13B). It is still not known how *STAT1* GOF cells deteriorate the function of STAT3.

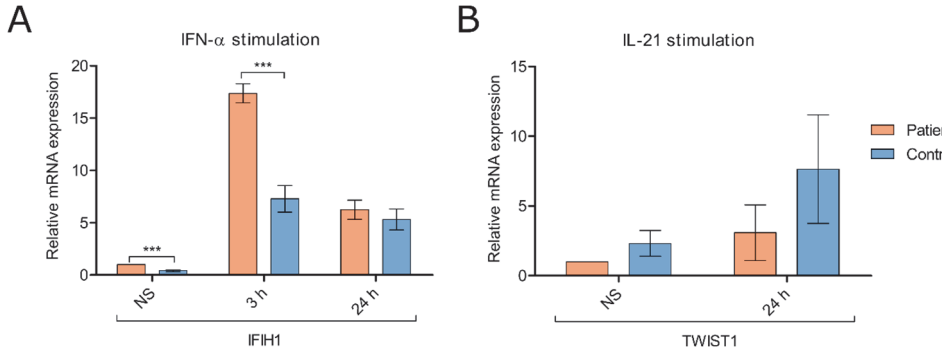

Figure 13. The expression of the target genes of STAT1 and STAT3. (A) STAT1 target gene *IFIH1* gene expression was detected in unstimulated (NS) and IFN-α stimulated PBMCs after 3 and 24 h. (B) STAT3-dependent *TWIST1* gene expression was measured in unstimulated (NS) and IL-21 stimulated cells after 24 h. The total RNA was purified using RNeasy Micro Kit (Qiagen) and reverse-transcribed into cDNA. The relative gene expression levels were calculated using the ΔΔCt method and normalized to the expression of housekeeping gene β-ACT. Results were from 4 independent experiments (n=4 patients and n=7 controls). Statistical significance was assessed by the unpaired two-sample t-test using Graphpad Prism software, *** $p < 0.001$.

According to several previous studies, the phosphorylation kinetics of STAT3 is not disturbed (Bloomfield et al., 2018; Zhang et al., 2017; Zheng et al., 2015). Our results reflected the same effect (Fig. 14A and B). However, we saw a clear impact on the balance of STAT factors. The *STAT1* GOF variant impaired the balance of STAT1 and STAT3 phospho-forms in the cell (Fig. 14C and D). In patients, IFN-α stimulation resulted in increased and prolonged dominance of p-STAT1 over p-STAT3 (Fig. 14C). In IL-21 stimulated control cells, the p-STAT3/p-STAT1 ratio curve inclined significantly during the first 30 minutes of stimulation and started to decline after 1 hour. But remarkably, the curve remained flat in patient cells (Fig. 14D).

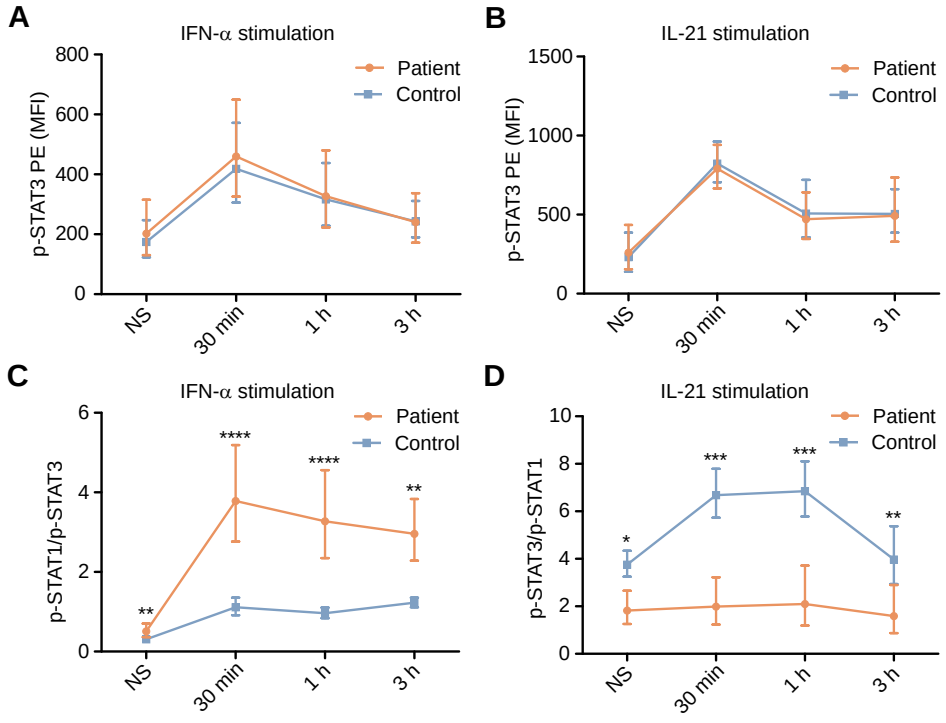

Figure 14. The balance of p-STAT3 and p-STAT1 in *STAT1* GOF patients. The phosphorylation of STAT3 was measured with flow cytometry in PBMCs that were left unstimulated (NS) or stimulated with IFN-α (A) or IL-21 (B). Results are shown as geometric MFI ×÷ geometric standard deviation from 4 patients and 7 controls (four independent experiments). The MFI ratio of p-STAT1/p-STAT3 from IFN-α stimulated cells (C) and the MFI ratio of p-STAT3/p-STAT1 from IL-21 stimulated cells (D) in different time-points. Results were received from 4 independent experiments (n=4 patients and n=7 controls). Results are shown as the geometric mean ×÷ geometric standard deviation. Statistical significance was assessed by the unpaired two-sample t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Taken together, these results demonstrate that *STAT1* GOF cells did not affect STAT3 phosphorylation after IL-21 and IFN-α stimulation. Nevertheless, the GOF variant significantly disturbed the balance of p-STAT3 and p-STAT1.

6.1.6.IL-27 variant modulates *IRF1* and *STAT1* expression (Study II)

There are thousands of SNPs associated with various immune-related diseases. The functional role is known only for a small fraction of these variants. To study expression-associated genetic variants, Kasela et al. isolated CD4+ and CD8+ cells from the blood of healthy individuals and subjected the extracted DNA or RNA samples to a genome-wide mapping of genetic variation affecting the expression of genes involved in immune response. eQTL analysis identified a

common missense variant in *IL27* (rs181206[C], NM_145659.3:c.356T>C) that associated with lower expression of *IRF1* ($p=1.9\times10^{-10}$) and *STAT1* ($p=3.69\times10^{-16}$) in CD4+ T cells, and could be a causal disease variant for T1D (Kasela et al., 2017). Importantly, IL-27 is crucial for T cell differentiation and survival by signaling through the STAT1/STAT3 pathway (Villarino et al., 2004). After binding to ISRE, the signaling pathway induces transcription of several interferon-induced genes, including *IRF1* and *STAT1* (Platanias, 2005). To confirm the functional effect of the *IL27* rs181206[C] allele, we investigated the effect of the SNP on *IRF1* and *STAT1* expression in human PBMCs. The cDNA variants of the IL-27 wild-type and missense (NM_145659.3:c.356T>C), as well as EBI3, were cloned. After transfection into HEK293 cells, the cell supernatants containing either IL-27 wild-type or the mutant protein were combined with an equal amount of EBI3 protein. To study the effect on *IRF1* and *STAT1* expression, we conducted an experiment using real-time qPCR in human PBMCs from four controls.

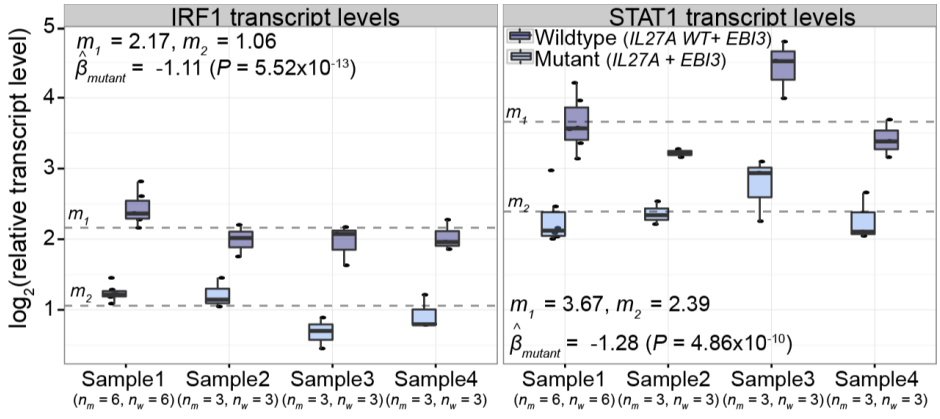

Figure 15. The impact of the missense p.Leu119Pro and wild-type variant of IL-27 on the expression levels of *IRF1* and *STAT1*. The log2 relative transcript levels (y-axis) are shown as a boxplot per allele and sample, with four samples in total. Every sample was run in multiple parallel reactions, indicated by n_m (number of mutant reactions) and n_w (number of wild-type reactions). Sample1 was used in two parallel sets. The mean expression in each class is shown by the grey dashed lines, where m_1 (wild-type) and m_2 (mutant) indicate the mean among samples. The effect of the mutant SNP on transcript levels was evaluated by linear mixed effects models.

We thereby demonstrated that the *IL27* mutant allele results in significantly lower transcript levels of STAT1 and IRF1 (Fig. 15). The missense variant that causes the amino acid Leu119Pro change in alpha-helical domain in IL-27 induced lower STAT1 and IRF1 transcript levels compared to the IL-27 wild-type. The comparison of the fixed effects of the rs181206[C] allele resulted in highly significant estimates – STAT1 with ^b mutant=–1.28 ($p=4.86\times10^{-10}$) and ^b mutant=–1.11 ($p=5.52\text{x}10^{-13}$) for IRF1. Taken together, this result indicates that the p.Leu119Pro of the *IL27* gene was the causal variant of these associations.

6.2. IL-22 and APECED (Study III)

6.2.1. APECED patients have decreased MAIT cell proportions

It is known that APECED patients have a severe deficiency of Th22 cells in their blood circulation and skin (Kisand et al., 2010; Laakso et al., 2014). Therefore, we enquired if the proportions of unconventional T cells, which are known for their IL-22 production capability, are also impaired in these patients. We conducted flow cytometry analysis to enumerate these cells (Fig. 16) (gating strategy displayed in Paper IV, Supplementary Fig. 1). We found that Vδ1+ and Vδ2+ γδ T cell proportions were not significantly altered, in accordance with previous studies (Tuovinen et al., 2009), nor did the iNKT cell numbers differ between patient and control samples. Nevertheless, among T cells, the percentages of MAIT cells (Vα7.2 TCR + and CD161+) were lower in patients than in controls ($p < 0.05$).

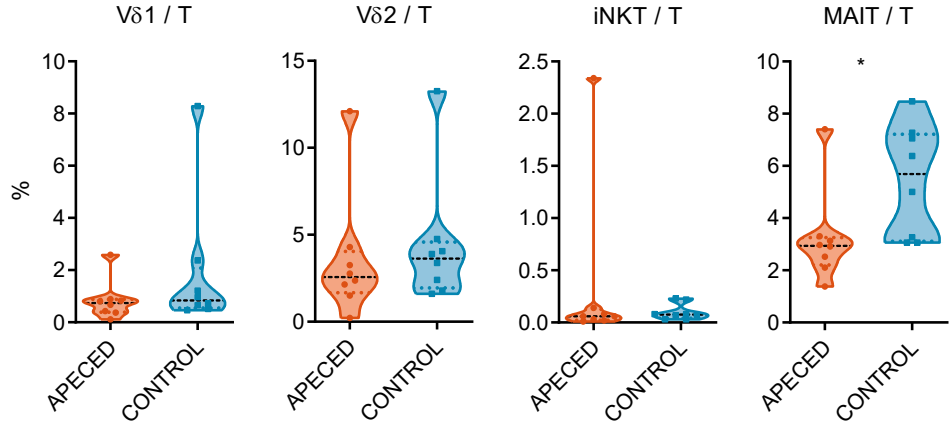

Figure 16. Comparison of the circulating lymphocyte subpopulations in 8 APECED patients and 8 age-matched controls. The surface marker expression was assessed on PBMCs by flow cytometry. The percentages of Vδ1+ and Vδ2+ γδ T cells, iNKT and MAIT cells were compared from the total T cell pool between patient and control sample groups. The outliers for different cell types did not overlap, apart from the APECED patient who had the highest proportion of both γδ T cell subtypes. In the violin plot, the width of the distribution of points is proportionate to the number of points in the value of the sample. The black discontinuous line represents the median, and the colored discontinuous line represents quartiles. Statistical significance was assessed using the unpaired t-test using R statistical software, * $p < 0.05$.

6.2.2. Saliva from APECED patients contains cytokine autoantibodies and reflects inflammation

IL-22 cytokine and MAIT cells are both important players in mucosal protection and homeostasis. Therefore, we analyzed saliva samples from APECED patients and controls to find signs of perturbations on the oral mucosa. First, we tested for the presence of cytokine autoantibodies from the saliva and corresponding plasma samples (Fig. 17). Importantly, anti-IL-22 and anti-IFN-α autoantibodies were present in saliva from all 10 APECED patients. Anti-IL-17F and anti-IL-17A autoantibodies from saliva were detectable at very low levels in a fraction of the patients who tested positive for corresponding circulating autoantibodies. In addition, we detected lipocalin (LCN)1 autoantibodies in the plasma of 3 of 13 APECED patients.

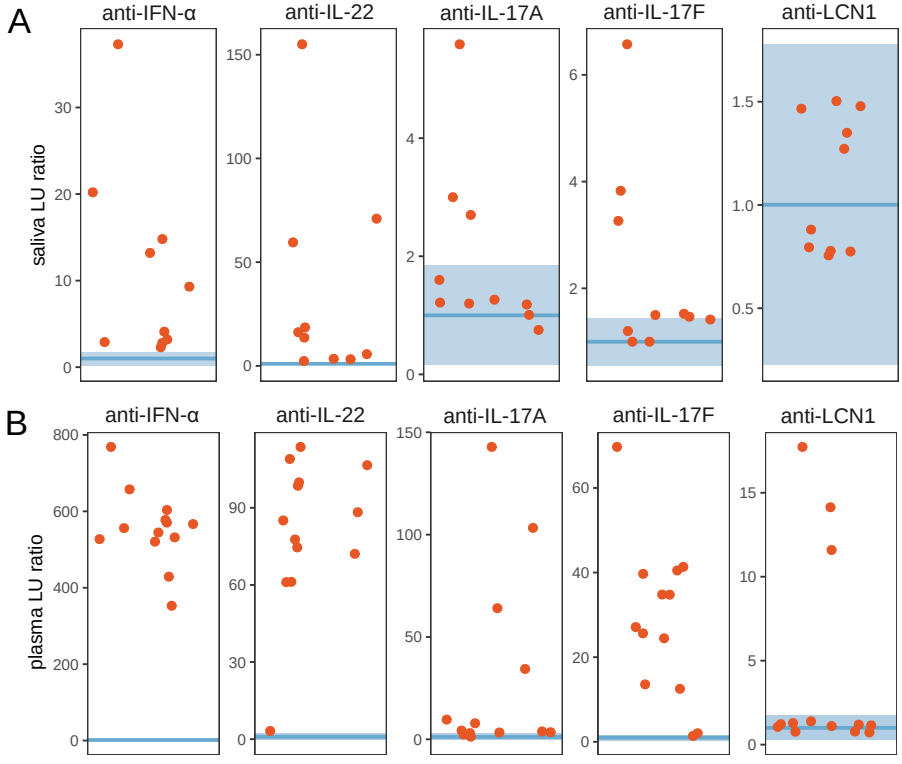

Figure 17. Autoantibodies from saliva and plasma of APECED patients and controls. The LIPS assay was conducted on the saliva (10 patients and 10 controls) (A) and plasma samples (13 patients and 7 controls) (B). The results are expressed as a LU ratio representing the fold over the mean of the control samples. The horizontal blue line represents the geometric mean of the LU of the control sample group. The transparent blue area shows the normal range, e.g. ± 3 standard deviations of the geometric mean.

The most striking protective function of IL-17 and IL-22 is the induction of an epithelial innate immune response directed against extracellular pathogens. One mechanism is by inducing the production of AMPs, such as S100 proteins (Wolk et al., 2006). Therefore, we also tested for different anti-S100A subtypes (S100A7A, S100A7, S100A8, S100A9) in the plasma, but there was no difference between the APECED patient group and the control group (Fig. 18).

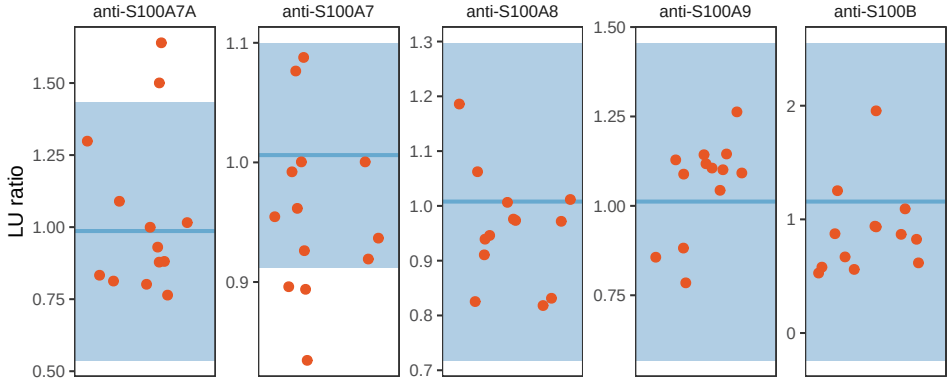

Figure 18. S-100A autoantibodies detected from plasma of APECED patients and controls. The LIPS assay was conducted on the plasma samples (13 patients and 7 controls). The results are expressed as a LU ratio representing the fold over the mean of the control samples. The horizontal blue line represents the geometric mean of the LU of the control sample group. The transparent blue area shows the normal range, e.g. ± 3 standard deviations of the geometric mean.

Collectively, these findings suggest that the local presence of IL-22 and IFN-α autoantibodies can aggravate the deficiency of these cytokines on mucosal surfaces.

In addition, we compared the concentration of various cytokines in APECED patients with those in controls from saliva (Paper IV, Supplementary Table 5). In the majority of the samples, IL-22, IL-17F, and IL-17A levels remained undetectable. However, pro-inflammatory cytokines IFN-γ, GM-CSF, and TNF-α were all significantly increased in APECED patients compared to the control group (Fig. 19A). This increase was indicative of local inflammation in the oral cavity, as the difference was not recapitulated in the plasma samples (Fig. 19B). Only CXCL10 was significantly increased from the tested plasma cytokines in patients, confirming an earlier report (Kisand et al., 2008).

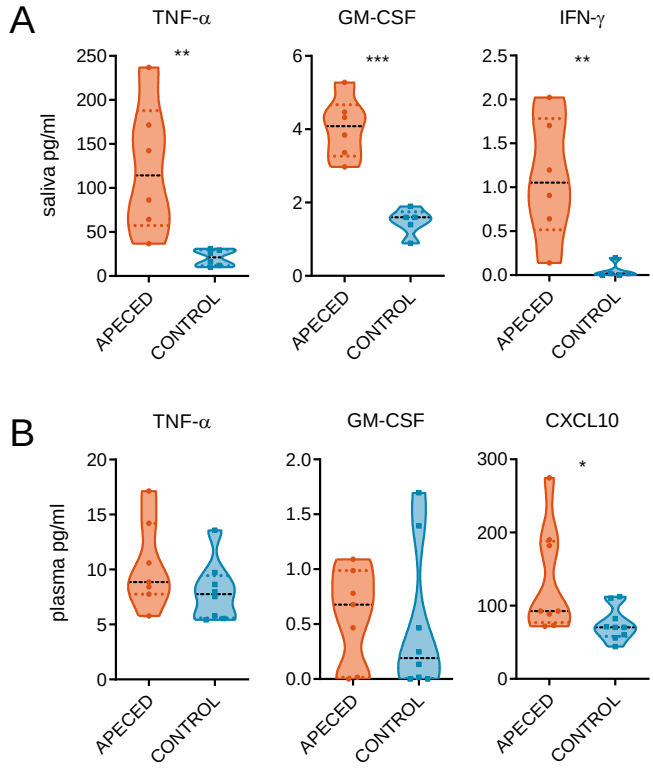

Figure 19. Cytokine quantification from APECED patients and controls. The cytokine measurement assay was conducted on the saliva (6 patients and 5–6 controls) (A) and plasma samples (7 patients and 8–9 controls) (B). In the violin plot, the width of the distribution of points is proportionate to the number of points in the value of the sample. The black discontinuous line represents the median, and the colored discontinuous line represents quartiles. Statistical significance was assessed with the unpaired t-test using Graphpad Prism software, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

As APECED patients are known to suffer from chronic mucocutaneous candidiasis on their mucosa, the inflammation could result from local infection. Therefore, we quantified *Candida* concentration (CFU/ml) in saliva from APECED patients and controls (Fig. 20). The results were compared to *STAT1* GOF and *STAT3* LOF patients, which are known to have high concentrations of *Candida* in the saliva. Only one APECED patient had CFU values significantly over the heathy control level.

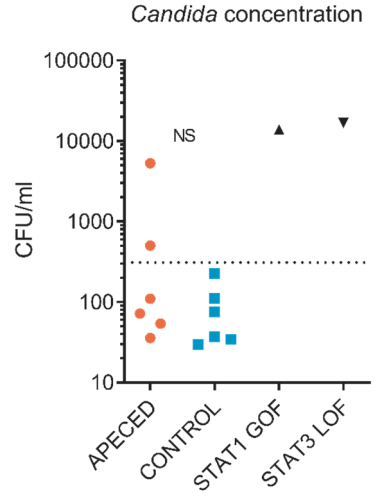

Figure 20. The concentration of *Candida* (CFU/ml) in saliva. The concentration was measured in 6 APECED patients, 6 control samples and as a positive control in two patients with different monogenic diseases with CMC – *STAT1* GOF and *STAT3* LOF. DNA of *C. albicans* from saliva samples and the cultured laboratory strain SC5314 was extracted. The concentration of *Candida* cells in patient samples was calculated based on the calibration curve constructed according to the Ct values of serially diluted *C. albicans* probes (starting from 2E7 CFU/ml). The normal range of the concentration of *Candida* was calculated based on values of control subjects: mean + 3 standard deviations (dotted line). The statistical significance was assessed with the unpaired t-test using Graphpad Prism software. NS – not significant.

Furthermore, correlation analysis was conducted to compare the *Candida* concentrations with significantly increased cytokine levels and autoantibody levels from patient saliva samples (Fig. 21). There was no correlation between *Candida* CFU values and pro-inflammatory cytokine concentrations. The only significant correlation was between *Candida* CFU and salivary anti-IL-17A levels, but this was caused by the one outlier – the patient with the highest *Candida* CFU value had the highest IL-17A antibody level (Fig. 21).

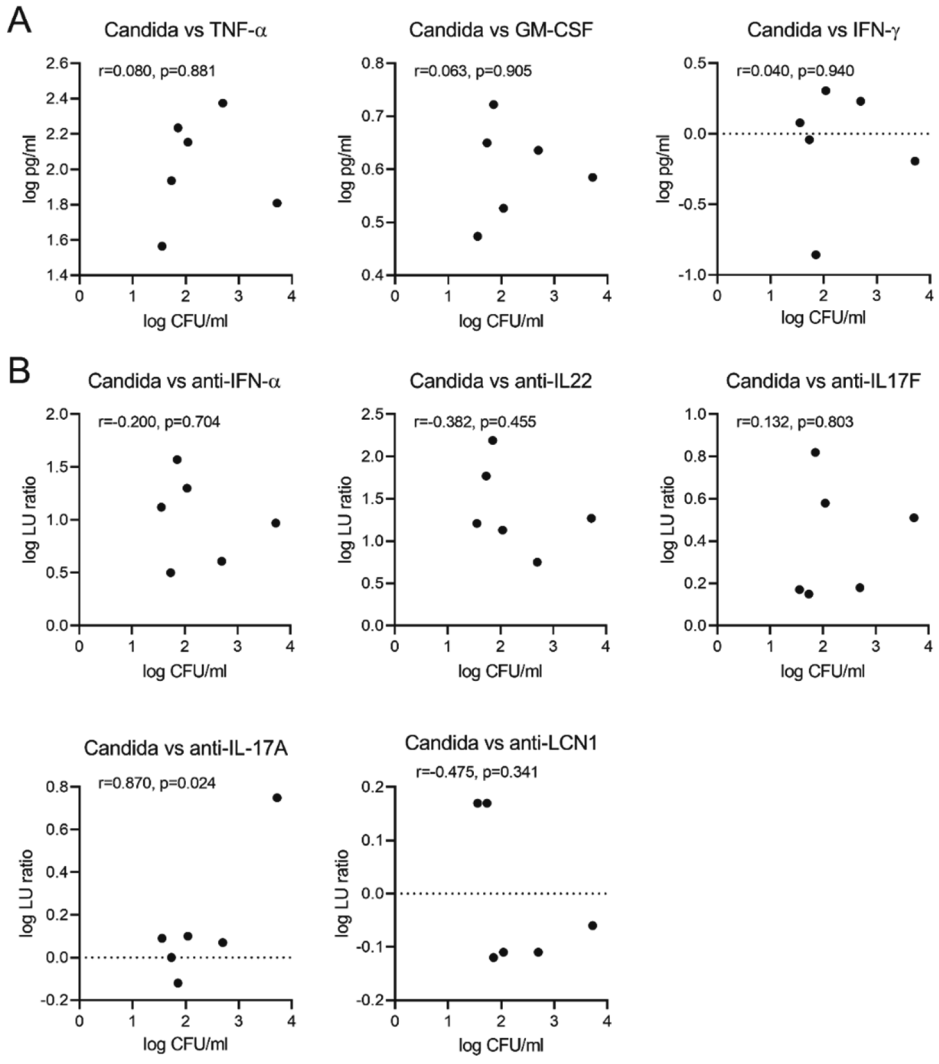

Figure 21. Correlation analysis between the *Candida* concentration (CFU/ml) and significantly increased cytokines (A) and autoantibody levels (B) from saliva. The measurements were done in 6 APECED patient samples. Values were log-transformed.

6.2.3. Buccal biopsy transcriptomes reveal alterations in APECED oral mucosa

Next, we studied gene expression in APECED oral mucosa. The gene expression analysis was conducted on 4 patients and 2 control buccal biopsy samples. Differentially regulated genes were identified based on the diffScores of gene expression (Paper IV, Supplementary Table 6). Seventeen significantly upregulated and ninety-one significantly downregulated genes were identified. Among the upregulated genes, several transcripts were associated with tumorigenesis (*DDR2, SHIP2, FANCG, SNHG7*). In contrast, genes important for epithelial barrier function (*SPRR2C, SPRR2B, TGM5*) and several AMP genes (*DEFB103B, DEFB103A, S100A12*) were downregulated. The significantly downregulated genes were further analyzed with the g:Profiler database. The mitotic cell cycle was identified based on annotation analysis as an associated biological process. Additionally, the gene lists with fold changes were analyzed by Ingenuity Pathway Analysis. We conducted a diseases and functions analysis, which predicted the cellular processes and biological functions affected by gene expression. The main terms that emerged related to cell cycle and mitosis (Table 6). In addition, the cell cycle process was predicted to be inhibited in patients (p-value 3.5E-08, activation z-score –2.256, the activation state was predicted to be decreased).

Next, we carried out a network analysis, which predicts genes that interact with each other (Paper IV, Supplementary Table 8). We focused on the network with the highest score, which consisted of 23 focus genes from our list and 12 interconnecting genes. The main network included the following biological terms – antimicrobial response, inflammatory response, and cellular function and maintenance (Fig. 22 and Paper IV, Supplementary Fig. 5).

Finally, we conducted an upstream regulator analysis, which predicts the activity state of regulators. The analysis correlated observed gene expression from the list with reported effects from the literature. The upstream regulator gene controls the expression of target genes in the dataset through participating regulators. The top two master regulators, transcription factor IIH (TFIIH) and aryl hydrocarbon receptor (AHR), were identified in the dataset based on the highest p-value of overlap (Paper IV, Supplementary Table 9, and Supplementary Fig. 6). TFIIH is an important protein complex with many biological roles, ranging from DNA repair to transcription to cell cycle regulation (Rimel & Taatjes, 2018). AHR is highly expressed by Th17 cells, and activation of AHR results in the expansion of Th17 cells and enhanced production of Th17 cytokines, particularly IL-22 (Monteleone et al., 2011; Veldhoen et al., 2008).

Taken together, the gene expression array results hinted that the pathways controlled by IL-22 may be downregulated in the buccal biopsy samples of APECED patients.

Table 6. Diseases and functions annotation top 10 of significantly downregulated genes of APECED patients. The core analysis was carried out on the reference set Ingenuity Knowledge Base. The settings included direct and indirect relationships between genes by default, 35 genes per network, 25 networks per analysis. The diseases and functions analysis was conducted, which predicted cellular processes and biological functions based on gene expression. The p-values were calculated using a Right-Tailed Fisher's Exact Test.

Categories	**Function**	**Diseases or function annotations**	**P-value**	**Activation z-score**	**Number of genes**
Cell cycle	Cell cycle progression	Cell cycle progression	3.57E-08	–2.256	23
Cellular function and maintenance	Endocytosis	Endocytosis by cervical cancer cell lines	6.23E-06	–0.628	5
Cell cycle	Interphase	Interphase	7.19E-06	–1.633	16
Cell cycle	Mitosis	Mitosis	1.78E-05	–0.876	12
Cancer, organismal injury and abnormalities	Breast or colorectal cancer	Breast or colorectal cancer	2.25E-05		55
Cellular assembly and organization, cellular function and maintenance	Formation	Formation of artificial clathrin cages	3.18E-05		3
Cell cycle	Mitosis	Mitosis of tumor cell lines	6.52E-05		6
Cell cycle	Mitosis	Mitosis of cervical cancer cell lines	1.13E-04		5
Cell cycle	Interphase	Interphase of tumor cell lines	1.16E-04	–1.342	10
Hematological system development and function, hematopoiesis, tissue morphology	Quantity	Quantity of short-term hematopoietic stem cells	1.52E-04		2

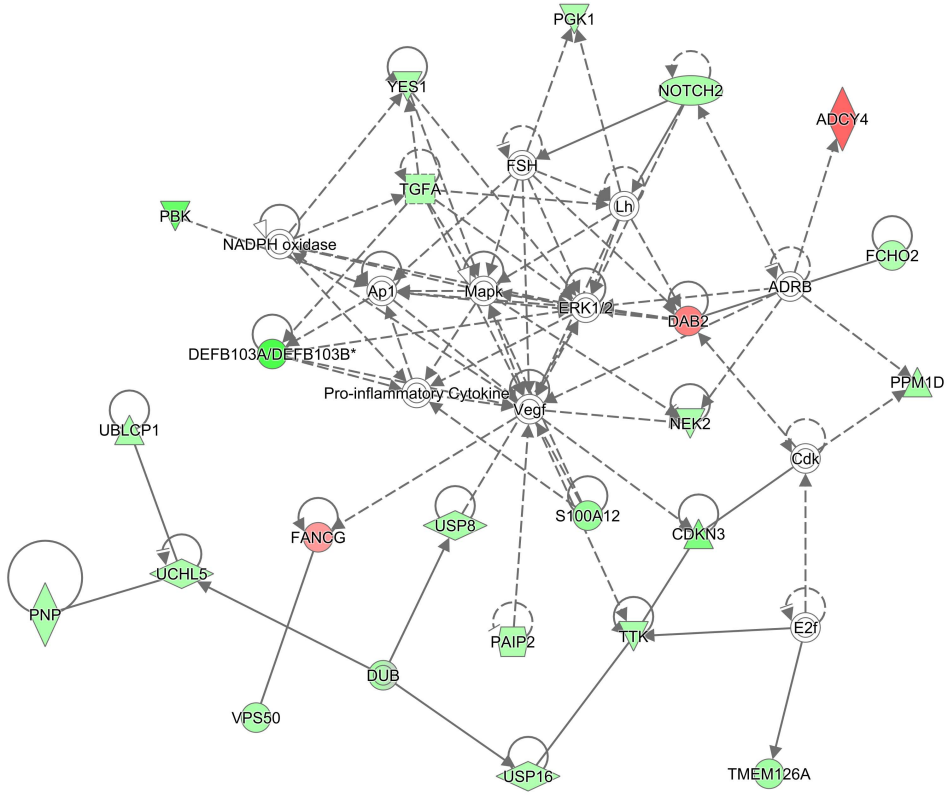

Figure 22. The IPA network diagram of differentially expressed genes in APECED patient versus control buccal biopsies. Gene expression analysis was conducted on buccal biopsy samples of 4 APECED patients and 2 controls. The network analysis with the highest score consisted of 23 focus genes and 12 interconnecting genes. The network with the highest score (score 51) was categorized by IPA function – "antimicrobial response, cellular function and maintenance, inflammatory response." The intensity of the gene color indicates the degree of upregulation (red) or downregulation (green) of a given gene. The legend of figure shapes and relationships can be found in Paper IV, Supplementary Fig. 5.

6.2.4. Salivary microbiota analysis is consistent with dysbiosis in the oral cavity of APECED patients

We hypothesized that IL-22 paucity could impact the microbial community in the oral cavity. Therefore, we analyzed the salivary microbiotas in 6 APECED patients and 6 age-matched controls. The microbial communities were significantly different in APECED patients on the whole-community (beta-diversity) level (Fig. 23) in comparison to controls. All differences were significant using a permutational ANOVA test (ASV R^2=14.6%, p=0.017, species R^2=20.2%, p=0.036, genus R^2=23.5%, p=0.021). Interestingly, the two patients with elevated *Candida*

levels in their saliva samples (Fig. 20) clustered together at the genus and species level (Fig. 23).

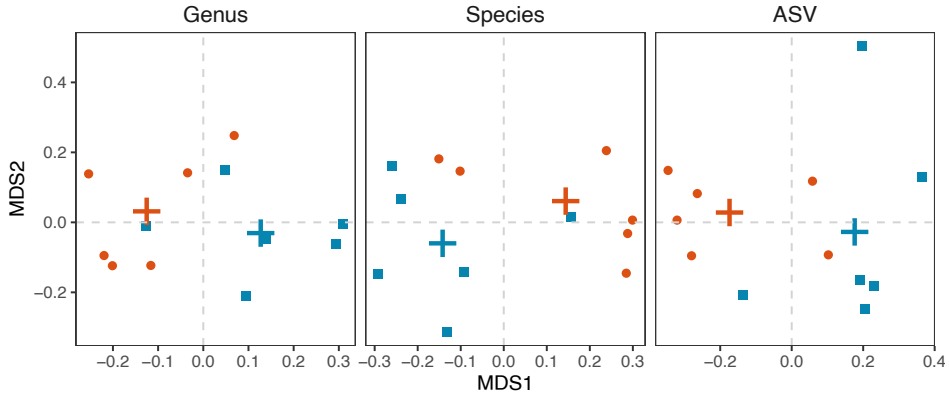

Figure 23. The beta-diversity of the salivary microbiota. Microbiotas from salivary samples were analyzed in 6 APECED patients and 6 controls. Multidimensional scaling was applied based on Bray-Curtis distances with first and second coordinates depicted for the genus, species, and ASV levels. Each point represents the microbiome of one individual. Orange dots – APECED patients, blue dots – controls. Crosses represent centroids per group. Black boxes – patients with high *Candida* concentration of saliva samples compared to the normal range of *Candida* concentration (values of control subjects mean + 3 standard deviations).

Next, we analyzed the taxonomic groups. The most abundant genus in both patients and controls was *Streptococcus* (Fig. 24). A significantly higher abundance of *Fusobacterium* ($p < 0.05$) was revealed in APECED patients compared to the control group. In addition, a relatively rare genus, *Lachnoanaerobaculum*, was more abundant in controls.

We further analyzed the taxonomic groups, species, and ASV (amplicon sequence variant). At the species and levels, we noted a significantly lower abundance of *Streptococcus salivarius* ($p < 0.05$) in APECED patients than controls.

In addition, we conducted an alpha-diversity analysis, which estimates bacterial diversity within a sample (Fig. 25). Three different indices were used to estimate counts of observed and unobserved species – the Chao 1 Estimator, observed taxa with abundance over zero, and the Shannon Diversity Index. Interestingly, significantly increased Shannon diversity in patient samples was found at the species ($p < 0.05$) and ASV ($p < 0.01$) levels. Other alpha-diversity indices showed similar tendencies, but in this small dataset, the differences were not significant.

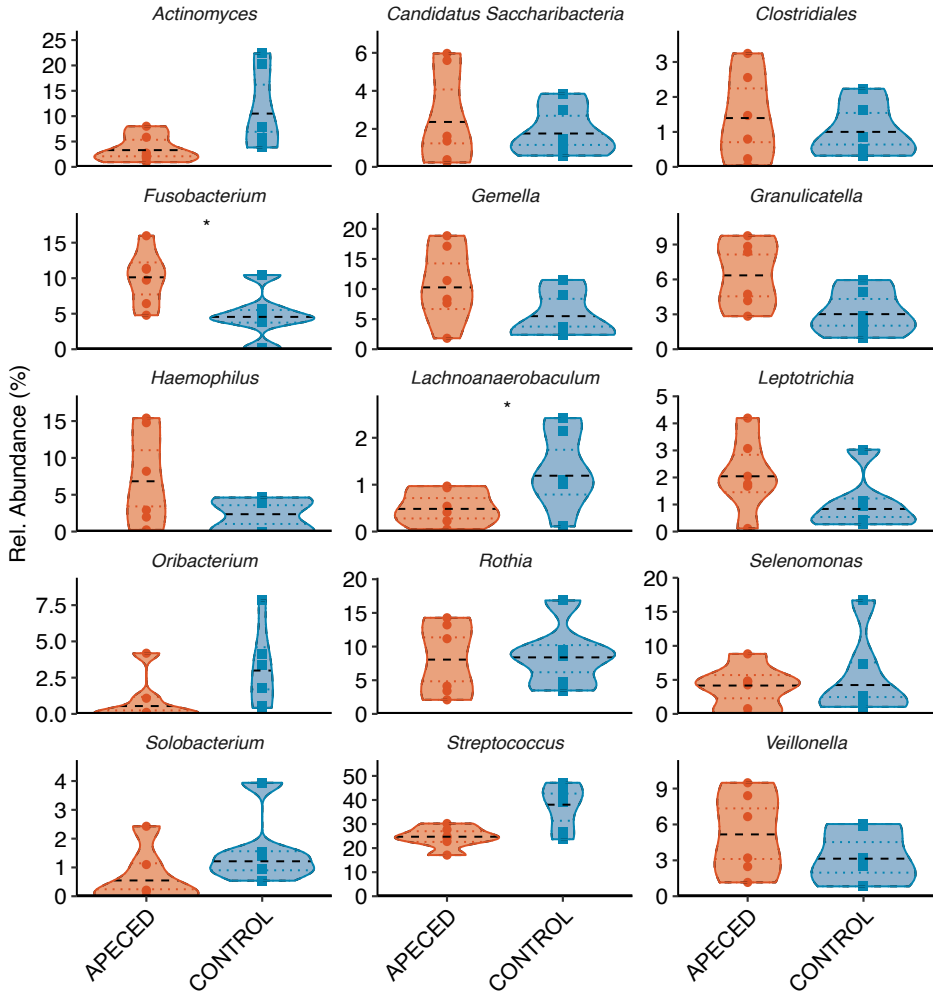

Figure 24. Profiles of the relative abundance per group in studied groups at the genus level. The microbiotas from salivary samples were analyzed in 6 APECED patients and 6 controls. Analysis was performed using the Wilcoxon rank-sum test using only taxa with median abundance > 100 sequence counts (0.5% relative abundance) and prevalence > 0.3 (present in at least 4 samples), * $p < 0.05$.

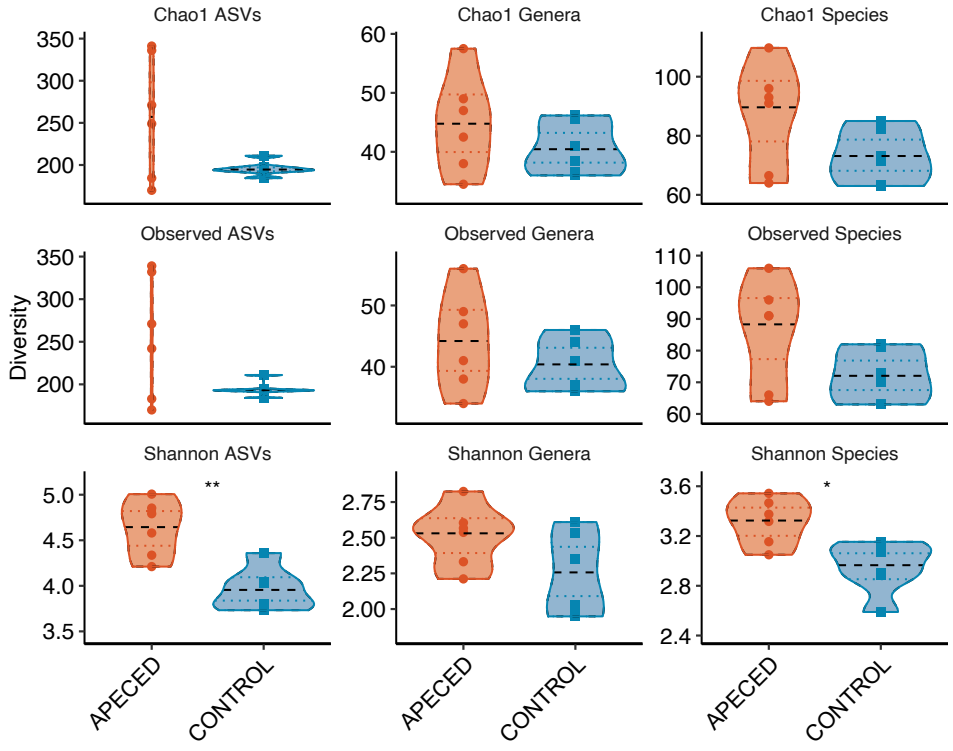

Figure 25. The alpha-diversity analysis of salivary microbiotas. Within-sample, the bacterial diversity was analyzed on the ASV, genus, and species levels in 6 APECED and 6 control samples using three different diversity indices, as indicated on the plots. * $p < 0.05$, ** $p < 0.01$. Chao 1, Chao 1 Estimator; Observed, observed taxa with abundance over zero; Shannon, Shannon Diversity Index.

Taken together, the results of the microbial 16S sequencing analysis indicated alterations in the oral microbiotas of APECED patients. The microbial communities were significantly different in APECED patients on the whole-community (beta-diversity) level. APECED patients exhibited an increased alpha-diversity at the ASV and species levels in comparison to controls.

7. GENERAL DISCUSSION

7.1. IFN-α and ISG in the pathogenesis of *STAT1* GOF disease

It has been shown before that after the stimulation of *STAT1* GOF cells with IFNs *in vitro*, ISGs are induced to a significantly higher extent in patient cells (Al Shehri et al., 2015; Mizoguchi et al., 2014; Zheng et al., 2015). This is confirmed by our results, which found that the ISG expression in circulating blood cells significantly increased in patients. To correlate the ISG levels to circulating IFN-α concentrations, we used the digital ELISA method, which was previously applied to characterize type I interferonopathies (Rodero et al., 2017), thus permitting direct comparison of cytokine concentrations. We showed that in comparison to patients who have defects in nucleic acid metabolism, the circulating levels of IFN-α in *STAT1* GOF patients were lower and not constantly elevated. IFN-α serum concentrations correlated well with the ISG gene expression level in blood cells and showed significant elevation from the control levels, even if the IFN-α concentration was only slightly over the lower limit of detection. Importantly, the IFN signature was evident even in the patient with undetectable IFN-α levels. Although most of the ISGs are upregulated by type I as well as by type II IFNs, some genes, such as CIITA, are preferably induced by IFN-γ (Der et al., 1998). We therefore consider it unlikely that IFN-γ has an impact on the IFN signature in our patients, as the CIITA expression was not elevated in any of our patients. Nevertheless, the controversy between the unstable level of IFN-α and concurrent strong ISG expression raises some questions, which could warrant further studies.

It is fascinating that the expression of ISGs is increased remarkably in APECED patients even at low IFN-α levels. Although we lack direct evidence, it is plausible that the hyperphosphorylation of p-STAT1 and high ISG expression are associated with its prolonged binding to chromatin target sites. Therefore, we wanted to study chromatin accessibility in *STAT1* GOF patients. The epigenomic landscape is shaped during development and is remodeled in response to environmental cues that organize the three-dimensional structure of the genome. In this respect, it is important to note that several members of the STAT family have been proposed to control the active state of enhancers and chromatin accessibility to master transcription factors (Vahedi et al., 2012; Wei et al., 2010). In addition, it has been shown that IFNs induce extensive remodeling of the epigenome landscape (Kamada et al., 2018; Park et al., 2017; Qiao et al., 2013). In line with these results, the ChIP-seq data from the current study show that the permissive chromatin mark H3K4me3 was significantly more enriched near ISGs in patient PBMCs than in control cells, suggesting that while binding to chromatin *STAT1* GOF variant promotes epigenetic changes compatible with higher gene expression and elevated reactivity to type I IFNs. The epigenetic memory of previous encounters has been described as trained innate immunity (Crișan et al., 2016; Netea et al., 2016), and exposure to the innate immune mediators, such as type I IFNs, may have effects analogous to those of BCG or β-glycan, although at

different chromatin sites (Arts et al., 2018). Therefore, the possible mechanism relies on the IFN signature in *STAT1* GOF patients, which is epigenetically determined.

It has been reported that *STAT1* GOF disease causing variants and monogenic interferonopathies occasionally share certain phenotypic features such as intravascular calcifications (Smyth et al., 2018; Uzel et al., 2013) and SLE-like disease (Rodero & Crow, 2016; Toubiana et al., 2016), which has provoked the idea that *STAT1* GOF disease could be classified as a type I interferonopathy (Al Shehri et al., 2015). Arterial calcification is a feature of type 1 interferonopathies that is thought to occur due to endothelial damage by constantly elevated type I IFN levels. Aortic calcification had only been described in *STAT1* GOF patients (Smyth et al., 2018; Uzel et al., 2013). It has been suggested that an increased IFN signal underlies the pathogenesis of this vascular abnormality (Smyth et al., 2018). In addition, there was a very recent report on a novel *STAT1* GOF variant (c.1398C>G, p.Ser466Arg) of which the phenotype resembled interferonopathy. The patient features brain calcification, arthritis, recurrent pericarditis, leukopenia, and thrombocytopenia (Stellacci et al., 2019), representing a remarkable clinical overlap between type 1 interferonopathies and *STAT1* GOF disease. Nevertheless, in comparison to patients who have interferonopathies with high IFN-α levels, the circulating levels of IFN-α in *STAT1* GOF patients were lower and not constantly elevated.

Around 37% of *STAT1* GOF patients develop autoimmune manifestations (Lorenzini et al., 2017; Toubiana et al., 2016), and *STAT1* GOF disease autoimmune features may result from excessive IFN-α signaling. We suggest that an aggravated response to type I IFNs predisposes *STAT1* GOF patients towards autoimmunity, which is a common feature of this patient group. The enhanced autoimmunity of patients with *STAT1* GOF disease causing variants is likely to result from stronger IFN-α signaling, as some of these autoimmune features are observed in patients treated with recombinant IFN-α (e.g., thyroiditis) and in patients with type I interferonopathies (e.g., SLE) (Crow, 2011; Crow & Manel, 2015). However, not every patient becomes affected by autoimmunity. Of the five patients who were recruited for this study, two suffered from autoimmune diseases, and one patient developed antinuclear autoantibodies. Of the two patients who were still spared from autoimmunity, one was a 9-year-old child who was potentially at the risk of developing autoimmunity in the future. Interestingly, the adult patient without any autoimmune features had the lowest ISG expression and an IFN-α concentration below the detection limit. To confirm that, among the *STAT1* GOF patients, higher IFN-α levels and ISG expression correlate with a higher rate of autoimmunity would require longitudinal studies in larger patient cohorts.

7.2. p-STAT1 and p-STAT3 balance is disturbed

STAT1 GOF patients have a dramatic decrease in the number of Th17 cells (Liu et al., 2011; Takezaki et al., 2012). It has been hypothesized that the differentiation and impaired function of Th17 in patients is associated with STAT3 transcription factor. One could presume that *STAT1* GOF variant impairs the function of STAT3, as *STAT1* GOF patients have decreased STAT3 target gene expression. The imbalance disrupts the transcription of genes by increasing STAT1 but decreasing STAT3-dependent gene transcription (Zheng et al., 2015). Our results corroborated this finding – after IFN-α stimulation of PBMCs, the STAT1 target gene expression was higher in patients, but the STAT3 target gene expression was lower after IL-21 stimulation in patients vs. controls. It has been shown that inhibition of STAT1 phosphorylation in patient cells with FLU rescues STAT3 activity (Zheng et al., 2015). However, the precise mechanism of how *STAT1* GOF variant interferes with STAT3 function is still under investigation.

Several hypotheses have been proposed to explain how *STAT1* GOF variant may hinder STAT3 activity. One could speculate that *STAT1* GOF version can interfere with the phosphorylation of STAT3 protein. However, the phosphorylation kinetics of STAT3 is not changed in *STAT1* GOF patients, according to several previous studies (Bloomfield et al., 2018; Zhang et al., 2017; Zheng et al., 2015). We also found that the p-STAT3 level was similar in patient and control PBMCs after stimulation with IFN-α and IL-21. Another proposed mechanism is that the mutated STAT1 has a stronger ability to pull STAT3 into a heterodimer complex (Platanias, 2005). However, a research group from the UK has demonstrated that *STAT1* GOF variant does not cause sequestration of STAT3 into STAT1/STAT3 heterodimers (Zheng et al., 2015). Possibly, the *STAT1* GOF variant is able to impair STAT3 binding to chromatin. This might be caused by the increased ability of the mutated STAT1 variant to bind to the target sequences of ISGs, which leads to an inhibitory capacity for STAT3 to bind to the target sequences (Hiller et al., 2018). A similar possibility has been suggested by Hu and Ivashkiv (Hu & Ivashkiv, 2009), hypothesizing that STAT1 is able to suppress the function of STAT3 by replacing STAT3 binding to the target sequences. However, some studies have demonstrated that *STAT1* GOF variant does not impair STAT3 DNA-binding to a STAT-consensus sequence (Zheng et al., 2015). However, in a ChIP assay, lower STAT3 occupancy of the proximal cFos promoter region was demonstrated in patient and control cells stimulated with IFN-α, suggesting that endogenous STAT3 DNA binding may be reduced (Zheng et al., 2015). Other plausible mechanisms have been considered. This includes the idea that the STAT1β isoform may inhibit transcription because it lacks the transcription activation domain. Also, the ability of *STAT1* GOF variant to recruit some suppressor-protein, inhibiting STAT3 to the target gene promoter, has been considered. However, so far there is not enough evidence to verify those hypotheses. Given the controversial results, the mechanism is not yet clear and needs further studies.

As many cytokines rely on the JAK-STAT signaling pathway, and several of them use overlapping STAT family members, the mechanisms that secure the specific effects of each cytokine have puzzled researchers for years. Our results suggest that the appropriate gene expression pattern after cytokine stimulation depends on the proper balance of different STAT phospho-forms that must follow precise dynamic changes.

7.3. Diagnostic tests for confirming *STAT1* GOF variants

Several diagnostic tests have been proposed in the literature to confirm the nature of novel *STAT1* GOF variants or to guide genetic testing of patients with CMC. Dhalla et al. developed an *ex vivo* assay for Th17 deficiency (Dhalla et al., 2016). However, a small fraction of *STAT1* GOF patients has normal Th17 numbers (Toubiana et al., 2016). The best confirmatory assay is the flow cytometric detection of p-STAT1 levels after IFN stimulation. This readout can be complicated due to large variations among patients as well as controls that lead to the overlap of the groups, as presented by Mizoguchi et al (Mizoguchi et al., 2014). In addition, there has been a report of missed *STAT1* GOF disease diagnosis due to false-negative Sanger sequencing of the *STAT1* gene. Because of the lack of availability of diagnostic resources at the time, no functional testing was performed (Hosking et al., 2020). This could have been avoided with a simple measurement of phosphorylated STAT forms. Our results suggest that the determination of the dynamics of p-STAT3/p-STAT1 levels after IL-21 stimulation leads to clear-cut discrimination between patient and control samples.

7.4. IL-27 in T1D susceptibility

Human genetic studies implicate IL-27 in the development of T1D, but the underlying mechanisms remain largely unknown. There have been conflicting results of the role of IL-27 in T1D, and some studies have suggested protective and some pathogenic impact (Barrett et al., 2009; Ciecko et al., 2019; Kasela et al., 2017; Plagnol et al., 2011; Wang et al., 2008).

A SNP rs4788084[T] on chr16p11.2 close to the *IL27* gene was found to be associated with protection against T1D in a GWAS (Barrett et al., 2009; Plagnol et al., 2011). Plagnol et al. discovered that *IL27* allele is associated with reduced positivity of a T1D associated autoantibody (Plagnol et al., 2011). A mouse study demonstrated that IL-27 signaling conferred protection against diabetes by inhibiting hyperglycemia and pancreatic islet inflammation (Fujimoto et al., 2011). On the contrary, some studies have investigated the potential pathogenic role of IL-27 in autoimmune diabetes. Wang et al. detected a high level of IL-27 in NOD mice, and the treatment of diabetogenic splenocytes with IL-27 accelerated the onset of the disease (Wang et al., 2008). In addition, another study

on mice discovered that IL-27-deficient NOD mice were completely resistant to T1D (Ciecko et al., 2019).

Our study found an even stronger trans-eQTL signal for a missense SNP rs181206[C] (Kasela et al., 2017) within the *IL27* gene compared to the SNP rs4788084[T] (Barrett et al., 2009; Plagnol et al., 2011), which could be a causal disease variant for the T1D association (Kasela et al., 2017). IL-27 is crucial for T cell differentiation and survival by signaling through the STAT1/STAT3 pathway (Villarino et al., 2004). After binding to ISREs, the signaling pathway induces the transcription of several interferon-induced genes, including *IRF1* and *STAT1* (Platanias, 2005). Our idea was to test the functional effects of IL-27 (rs181206[C]), where instead of the wild-type form, a missense variant leads to the p.Leu119Pro substitution. Our studies with the mutated form of IL-27 p.Leu119Pro, which was associated with protection against T1D, confirmed its decreased capacity to activate the STAT1 pathway. We demonstrated that the *IL27* mutant allele results in significantly lower transcript levels of STAT1 and IRF1. In conclusion, our experiment suggests that the rs181206[C] variant of the *IL27* gene confers protection against T1D. The IL-27 variant effect was specific to CD4+ T cells, as we did not detect significant trans-eQTL effects in this region in CD8+ T cells. Kasela et al. also found higher expression levels of the *IL27RA* and *IL6ST* (gp130) genes, which together act as a receptor for the IL-27 cytokine, in $CD4^+$ cells in comparison to $CD8^+$ T cells (Kasela et al., 2017).

IL-27 has pro-inflammatory and anti-inflammatory features. IL-27 drives inflammation by promoting the early commitment of naïve CD4+ T cells to a Th1-specific lineage through STAT1 (Lucas et al., 2003; Takeda et al., 2003). In addition, IL-27 inhibits inflammation by suppressing Th17 differentiation and inducing a Treg-like activity in differentiated Th1 and Th2 effector cells (Delgoffe et al., 2011). The anti-inflammatory effects of IL-27 signaling, which regulates polarization of T-cell subsets, and cytokine production have been demonstrated in animal models of experimental autoimmune encephalitis (EAE) (Batten et al., 2006; Stumhofer et al., 2006), allergic asthma (Miyazaki et al., 2005), and delayed-type hypersensitivity of skin (Miyazaki et al., 2008). It has been hypothesized that IL-27 represents a novel, promising target/agent for the treatment of RA, EAE, colitis, and psoriasis (Meka et al., 2015). Conversely, our results on T1D associations with IL-27 and several mouse studies have shown the pathogenic role of IL-27. Therefore, it is necessary to further study the conflicting role of IL-27 as a treatment for autoimmune diseases.

7.5. IL-22 paucity in APECED patients

Highly neutralizing autoantibodies against IL-22 and IFN-α are distinctive characteristics of APECED patients. These antibodies have potential biological implications. Previous studies have substantiated that due to the neutralization of the low physiologic levels of IFN-α, the blood cells of APECED patients have clearly reduced levels of IFN-induced gene expression compared with those of

controls (Kisand et al., 2008). Moreover, CMC in APECED patients is associated with the presence of circulating anti-IL-22 and IL-17F (Kärner et al., 2013; Kisand et al., 2011; Laakso et al., 2014) and with the inability of circulating and skin-populating CD4+ T cells to secrete IL-22 after their stimulation *in vitro*. As IL-22 is an important homeostatic cytokine for epithelial surfaces, its shortage can have additional consequences apart from CMC. Indeed, impaired mucosal barrier function has been suspected in APECED patients due to increased levels of antibodies to commensals (Hetemäki et al., 2016).

The cause for the almost complete absence of IL-22 production by circulating and skin CD4+ T cells in APECED patients is still not known. Furthermore, the role of other lymphoid cells with Th17-related cytokine secreting capacity is not yet clear in this disease. The association of iNKT cells with AIRE-deficiency has remained controversial (Ferre et al., 2016; Lindh et al., 2010; Pitt et al., 2008). Nevertheless, there is a previous report that IL-17A secreting γδ T cells are increased in APECED (Fujikado et al., 2016). According to our study, the percentages of Vδ1+ and Vδ2+ γδ T cells did not differ between patients and controls. However, we found significantly decreased proportions of circulating MAIT cells in APECED patients. MAIT cells are reportedly present in oral mucosa and are capable of secreting Th17 cytokines (Sobkowiak et al., 2019). Considering this, the paucity of MAIT cells may contribute to the shortage of IL-22 in mucosal surfaces. The paucity of MAIT cells can be connected to aberrations in the microbiota that produce metabolites necessary for MAIT cell development in the thymus (Legoux et al., 2019). In addition, Treiner et al. have shown that MAIT cells are not present in germ-free mice, indicating that commensal microbiota is required for their expansion in the gut lamina propria. Therefore, the reduction of MAIT cells in APECED refers to a possible microbiota change. The deficiency of MAIT cells in patients can also be related to the presence of highly neutralizing autoantibodies against type I IFNs in APECED because MAIT cells are activated by type I IFNs.

While the main producers of IL-22, distinct cell types from the lymphoid lineage (Parks et al., 2015), are deficient in APECED patients, neutrophils and dendritic cells are also able to contribute to the local or circulating concentration of IL-22 (Mann et al., 2014; Zindl et al., 2013). It is possible that the neutralizing effect of autoantibodies can deplete the remaining IL-22 from various sources. IL-22-neutralizing autoantibodies from APECED patients have been shown to possess potential pathogenicity for CMC, which was confirmed in a mouse model of oropharyngeal candidiasis (Bichele et al., 2018). Bichele et al. demonstrated that patient-derived monoclonal antibodies that cross-react with murine IL-22 increase the fungal burden on *C. albicans* infected mucosa (Bichele et al., 2018). Interestingly, we showed autoantibodies against IFN-α and IL-22 in patients' saliva samples. The levels of autoantibodies varied a lot between different patients. These local autoantibodies may further aggravate an IL-22 shortage; nevertheless, we need more proof to support this possibility. Neil et al. claim that IFN-α acts on intestinal epithelial cells to increase the proportion of macrophages and IL-22-producing innate lymphoid cells. The cells, in turn, promote p-STAT3

signaling in intestinal epithelial cells (Neil et al., 2019). As recently reported, one could also hypothesize that the local neutralization of IFN-α can further impair the IL-22 secreting cell recruitment (Neil et al., 2019).

7.6. Disturbed local AMP production and epithelial barrier

IL-22 is known to be an essential homeostatic cytokine on epithelial surfaces. IL-22 has similar and even synergistic effects with IL-17A, but it also exerts features to secure the barrier function of the epithelium (Costa et al., 2013; Eidenschenk et al., 2014; Monteleone et al., 2011; Rubino et al., 2012). More specifically, IL-22 together with IL-17A confers epithelial protection via the STAT3 signaling pathway by inducing the production of AMPs, such as S100A and LCN2 from epithelial cells (Cash et al., 2006; Zhang & Gallo, 2016). Therefore, considering the paucity of IL-22, the secretion of AMPs can be impaired in APECED patients. However, LCN2 concentration in the saliva of APECED patients was not impaired. Also, a previous study did not find decreased beta and alpha defensin levels in APECED saliva samples (Oftedal et al., 2017). On the contrary, our gene expression analysis of buccal biopsy samples revealed that *DEFB103B, DEFB103A,* and *S100A12* were significantly downregulated in the mucosa of APECED patients. But some other AMPs, like *S100A8* and *S100A9*, had similar expression in patients and controls in buccal biopsy samples. These results suggest specific, but not universal, impairment of AMP production in the APECED oral cavity. Interestingly, autoantibodies against AMPs, like LPO (Burbelo et al., 2019), DEFA5 (Dobeš et al., 2015), LCN1 (Fishman et al., 2017), BPIFA1, and BPIFA2 (Burbelo et al., 2019) have been described previously in APECED patients. In addition, we detected LCN1 autoantibodies in the plasma of three of 13 APECED patients included in this study but did not find autoantibodies against the S100A family of antimicrobial peptides. The fact that we did not detect S100A autoantibodies, but the expression level was downregulated, could hint that the lack of IL-22 could be the possible mechanism behind the impairment of AMP-producing cells.

Interestingly, our buccal biopsy samples' gene expression data suggested differences in the upstream and downstream molecular events of the IL-22 pathway. The analysis predicted regulators that caused changes in gene expression. Notably, the upstream regulator analysis identified AHR, which is the transcription factor necessary for IL-22 production (Alam et al., 2010). The regulator analysis hints that the production of IL-22 is disturbed in APECED mucosa. In addition, the network analysis revealed impaired antimicrobial response, cellular function and maintenance, and inflammatory response. Indeed, a mouse study has detected that AhR signaling via IL-22 inhibits inflammation and colitis in the gastrointestinal tracts of mice (Monteleone et al., 2011). Moreover, our "diseases and functions" analysis of the gene expression data predicted that processes like the cell cycle and mitosis are affected. Therefore, disturbed epithelial barrier function can also be suspected from the reduced expression of several genes

related to the mitotic cell cycle in patients. This can lead to defective wound healing on epithelial surfaces, which can become especially important during inflammatory processes. Importantly, developing an oral and esophageal squamous cell carcinoma has been associated as a susceptibility factor in APECED patients due to chronic inflammation of recalcitrant *Candida* infection (Rautemaa et al., 2007). In line with this, the patient saliva samples revealed significantly increased levels of pro-inflammatory cytokines. As IL-22 was recently shown to protect intestinal stem cells against genotoxic stress and thus against colon cancer (Gronke et al., 2019), we suggest that the susceptibility for oral and esophageal squamous cell carcinoma may result from the combination of persistent inflammation with the lack of the protective function of IL-22.

7.7. Salivary microbiota differs in APECED patients compared to controls

In addition to supporting epithelial barrier function, IL-22 is capable of shaping the gut microbiota (Fatkhullina et al., 2018). Considering the importance of the tight regulation of IL-22 levels to enable the symbiosis of the host with commensals (Bessman & Sonnenberg, 2016), we hypothesized that IL-22 paucity could impact the microbial community in the oral cavity. Indeed, the salivary microbiome of patients was significantly different from controls and showed higher diversity. The dysbiosis in APECED patients' oral cavity has been described in an earlier investigation with some overlaps in differentially abundant taxonomic units between the two studies, but also with discrepancies that can arise from various technical, analytical and biological differences between the two studies (Bruserud et al., 2018). Alterations in the microbiota could also be caused by medications, as well as Sjögren's-like syndrome, which is especially frequent among APECED patients in the US (Ferre et al., 2016). However, we think that neither factor had an impact on our analysis, as none of the study subjects received immunosuppressive drugs, and only one patient was on antifungals; the only other medications were hormone replacements. Moreover, objective sicca symptoms are rare in European APECED patients and were reportedly present only in patients over 33 years of age (Oftedal et al., 2017). Our study included relatively young patients (17.7 ± 10.9 years), and none of them reported dry mouth.

Candida infection itself is also a potential modifier of the microbiota. According to our beta-diversity analysis, the two patients with elevated *Candida* concentration clustered together at the genus and species level but did not differ from the other patients in any other studied parameter. CMC is also a possible inducer of pro-inflammatory cytokines that were elevated in the patients' saliva, which in turn can influence changes in the microbial communities. Although we did not see any significant correlation between *Candida* CFU values and cytokine concentrations, we cannot rule it out with our small study group. The cause of the salivary pro-inflammatory cytokine increase remains unknown. The oral cavity was

regularly examined by the attending pediatric endocrinologist, and no clinical signs of gingivitis and periodontitis were appreciated. The autoimmune attack towards mucosal antigens or preclinical inflammation in the salivary glands remains an option.

Whether susceptibility to candidiasis is primarily caused by the neutralizing autoantibodies to Th17 cytokines or by the impaired production of IL-22 and IL-17A is unknown. In most reported studies, the correlation between the presence of Th17 cytokine autoantibodies and CMC is ~70% (Constantine & Lionakis, 2019). Therefore, it is hypothesized that other immune mechanisms contribute to CMC in APECED. APECED patients have an altered immune response to *C. albicans*, including a dysregulation of IL-23p19 production in monocytes, which possibly contributes to the selective susceptibility to CMC (Bruserud et al., 2017). No APECED patients or controls had autoantibodies against IL-23 (Bruserud et al., 2017; Kärner et al., 2016). Ryan et al. describe monocyte-derived DCs from APECED patients as over-producing IL-2, IFN-γ, TNF-α, and IL-13, as we could see in our results with saliva, and demonstrated impairment in maturation in response to *C. albicans* (Ryan et al., 2008). However, the IL-23 response was comparable to that of the controls (Ryan et al., 2008). Thus far, the antifungal immune studies in APECED patients have focused on their systemic, not their mucosal, immune responses. Because the immune response requirements for effective mucosal versus systemic host defense against *Candida* are strictly segregated, it is likely that yet-unknown defects at the mucosal level may also contribute to CMC susceptibility in APECED patients (Constantine & Lionakis, 2019).

According to our results, APECED patients are characterized by alterations in their oral mucosa and increased diversity of their salivary microbiota, both of which can be, in theory, influenced by IL-22 shortage, possibly aggravated by the local action of autoantibodies and paucity of circulating MAIT cells.

8. CONCLUSIONS

Based on the studies given in this thesis, the following conclusions were made:

- IFN signature in *STAT1* GOF patients may be caused by epigenetic changes, as permissive chromatin mark H3K4me3 is enriched near interferon induced genes in patients' blood mononuclear cells. Impaired STAT3 dependent gene expression can be the result of impaired balance of p-STAT1 and p-STAT3 after cytokine stimulation.

- The missense variant in the *IL27* gene (rs181206[C]), associated with protection against T1D, which leads to an amino acid change p.Leu119Pro, reduce the capacity of the cytokine to upregulate *STAT1* and *IRF1*. This result indicates that IL-27 may promote autoimmunity towards pancreatic beta cells by enhancing the STAT1 signaling pathway.

- The IL-22 shortage in APECED patients may lead to an impaired barrier function and higher diversity of the local microbiome in their oral mucosa.

- The unique features of several monogenic diseases enable to dissect the precise role of cytokines in the human immune system.

9. SUMMARY IN ESTONIAN

Geneetilised variatsioonid mõistmaks STAT1/STAT3 signaaliraja tsütokiinide rolli immuunkaitses ning -patoloogias

Viimaste aastate jooksul on palju uuritud seoseid tsütokiinide ning erinevate autoimmuunhaiguste vahel, et leida tõhusaimaid diagnostilisi meetodeid ja ravivõimalusi. Monogeensed haigused on unikaalsed mudelid, et uurida erinevaid immuunsüsteemi mehhanisme. Antud uurimistöö keskendub kahele monogeensele haigusele. Mõlematel sündroomidel esineb krooniline kandidoos ning kahjustunud tsütokiinide funktsioon. Üks haigustest on põhjustatud dominantsest uudikfunktsiooniga (*gain-of-function*, GOF) mutatsioonist *STAT1* geenis, mille tulemusena areneb krooniline mukokutaanne kandidoos (CMC), ning teine autosoomsest retsessiivsest mutatsioonist *AIRE* geenis, mille tulemusena tekib autoimmuunne polüendokrinopaatia-kandidoos-ektodermaalne düstroofia (APECED) sündroom. Mõlemaid patoloogiaid ühendab vastuvõtlikkus kandidoosile, T-abistajarakkude alatüübi 17 tsütokiinide vähesus ning autoimmuunsed nähud. Sellegipoolest esineb ainult APECED patsientidel vähenenud I tüüpi interferoonide (IFN) vastus, mis teadaolevalt on põhjustatud vereringes leiduvate IFN-α vastaste autoantikehade neutraliseerivast toimest. Samas *STAT1* GOF patsientidel esinevad vaegused võivad olla põhjustatud ülemäärasest vastusest interferoonidele.

I tüüpi IFN-d omavad olulist rolli viirusinfektsioonide vastases kaitses. I tüüpi IFN-d aktiveerivad interferooni-stimuleeritud geenide (ISG) ekspressiooni läbi STAT1 signaaliraja. IFN-de liigne produtseerimine organismis võib tekitada erinevaid patoloogilisi seisundeid, sealhulgas vaskulaarseid haigusi ja autoimmuunsust. *STAT1* GOF patsientidel on teadaolevalt STAT1 transkriptsioonifaktor hüperfosforüleeritud seisundis ning STAT1 märklaudgeenid on üleekspresseeritud. Kuigi arvatakse, et antud mutatsioon inhibeerib transkriptsioonifaktori STAT3 funktsiooni, ei ole sellegipoolest täpne molekulaarne mehhanism veel teada. Lisaks ei ole siiani suudetud välja selgitada spetsiifilisi molekulaarseid signaaliradasid, mis põhjustavad *STAT1* GOF patsientidel vastuvõtlikkust autoimmuunsetele ilmingutele.

Interleukiin (IL)-27 on määrava tähtsusega T raku diferentseerumisel ja ellujäämisel, aktiveerides signaalirada samuti läbi STAT1 valgu. IL-27 omab kahetist rolli immuunsüsteemis – olenevalt kontekstist võib see olla põletikku soodustav või põletikuvastane. Ülegenoomsed assotsiatsiooniuuringud on tuvastanud *IL27* geenis rs4788084[T] alleeli, mis omab kaitsvat rolli 1. tüüpi diabeedi (T1D) vastu. Antud kaitsva omaduse täpne mehhanism ei ole siiani teada. Lisaks leidub varasemates töödes vastuolulist informatsiooni – kirjeldatud on nii IL-27 kaitsvat kui ka patogeenset rolli T1D puhul. Kuna IL-27 on välja pakutud potentsiaalse ravimina mitmete autoimmuunhaiguste korral, siis on äärmiselt oluline välja selgitada antud tsütokiini täpne mõju.

APECED on suurepärane mudel, mille kaudu uurida IL-22 puudulikkust. T-abistajarakkude alatüübi 17 rakud toodavad tsütokiine IL-22 ja IL-17A/F, mis soodustavad antimikroobsete peptiidide sünteesi. Lisaks sellele on IL-22-l oluline roll ka limaskesta kaitsebarjääris. Varasemates publikatsioonides on kirjeldatud, et APECED patsientidel esinev CMC on põhjustatud IL-22 ning IL-17F spetsiifiliste autoantikehade tõttu, mis omavad neutraliseerivat mõju antud tsütokiinide vastu. Lisaks omab rolli ka IL-22 tootvate rakkude puudulikkus. Suurem osa informatsioonist IL-22 funktsiooni kohta pärineb hiire ning *in vitro* eksperimentidest. Siiani ei ole teada, millised protsessid leiavad aset inimese suu limaskestas IL-22 puudulikkuse korral.

Uurimistöö eesmärgid:
Käesoleva töö põhiliseks eesmärgiks oli välja selgitada transkriptsioonifaktorite STAT1 ja STAT3 signaalirajas osalevate tsütokiinide rolli teatud monogeensete haiguste ja geneetiliste variatsioonide korral.

Spetsiifilisemad eesmärgid olid järgmised:

- analüüsida IFN-α ja IL-21 tsütokiinide mõju *STAT1* GOF variandiga patsientide lümfotsüütides;
- hinnata IL-27 missenssvariandi bioaktiivsust ning selle kaitsvat rolli T1D puhul;
- selgitada välja IL-22 tsütokiini puudulikkuse mõju APECED patsientide suu limaskestas.

Materjal ja metoodika:
Doktoritöös kasutati kolme erinevat uuritavate gruppi: 1) *STAT1* monogeense haiguse uurimistöös keskenduti viiele *STAT1* GOF patsiendile (vanuses 5–43 aastat); 2) IL-27 rs181206[C] geenivariandi uuring teostati, kasutades nelja kontrollisikut (vanuses 25–46 aastat); 3) APECED sündroomi uurimistöös hinnati 13 APECED patsienti (vanuses 4–55 aastat) Sloveeniast ning Eestist. Kõik teadustöös olevad isikud või nende eestkostjad allkirjastasid informeeritud nõusoleku.

STAT1 GOF patsientidel esinesid mutatsioonid *STAT1* geeni DNA-d seondavas või teisese heeliksi domeenis. Patsientidel esines lisaks CMC-le ka teisi komplikatsioone ning autoimmuunseid sümptomeid. *STAT1* GOF patsientide eksperimendid sooritati värske vere, plasma ja perifeerse vere mononukleaarsete rakkudega (PBMC). IFN-α valgu taset mõõdeti patsiendi seerumist digitaalse ELISA analüüsiga. Geenirekspressiooni mõõdeti qPCR meetodiga värskest verest ning tsütokiinidega IFN-α ja IL-21 stimuleeritud PBMC-dest. Rakkudest eraldati RNA, sünteesiti cDNA ning teostati reaalaja PCR, mille vahendusel määrati värskest verest ISG-de ning PBMC-dest STAT1 ja STAT3 märklaudgeenide ekspressioonitaset. Voolutsütomeetriga analüüsiti PBMC-des tsütokiinide IFN-α ja IL-21 toimet fosforüleeritud STAT1 ja STAT3 transkriptsioonifaktoritele ning värskest verest mõõdeti fosforüleeritud STAT1 taset. Avatud kromatiini uuri-

miseks genoomis kasutati kromatiini immunopretsipitatsiooni meetodit. Fragmenteeritud DNA sadestati uuritava antikehaga H3K4me3, mis on seotud avatud kromatiiniga, ning pretsipiteeritud genoomi fragmendid sekveneeriti.

Neljalt tervelt kontrollisikult eraldati IL-27 tsütokiini uurimiseks PBMC-d. Vererakke stimuleeriti IL-27 tsütokiinidega, mis olid metsiktüüpi järjestusega ning p.Leu119Pro missenssvariandiga. Seejärel sooritati geenieksрessiooni-analüüs STAT1 signaaliraja märklaudgeenidele *STAT1* ning *IRF1*.

APECED patsientidel esinesid mutatsioonid *AIRE* geenis. Lisaks CMC-le ilmnes patsientidel ka teisi komplikatsioone ning autoimmuunseid sümptomeid. Sealjuures ei esinenud ühelgi patsiendil *sicca* sündroomi ning neile ei sooritatud immunosupressiivset ravi. APECED patsientide eksperimendid viidi läbi värske vere, plasma, PBMC, suuõõne biopsia ning süljeproovidega. Voolutsütomeetriga detekteeriti limaskestaga-seotud invariantsete T (MAIT) rakkude tasemed. Lutsiferaasil põhineva immunopretsipitatsiooni analüüsiks kasutati patsientide plasmaning süljeproove, mille kaudu mõõdeti IL-22, IL-17A/F, IFN-α, LCN1 ja S100 subtüüpide vastaseid autoantikehasid. Erinevate tsütokiinide tasemeid mõõdeti plasma- ning süljeproovidest Luminex tehnoloogiaga. Suuõõne biopsia proovidest mõõdeti geenieksрessiooni tasemeid Illumina ekspressioonianalüüsi kiibi abil. Ekspressioonitasemeid analüüsiti seejärel IPA tarkvaraga kasutades GREAT andmebaasi. *Candida albicans*'i kontsentratsioon arvutati süljest isoleeritud DNA-ga läbiviidud qPCR abil. Lisaks sooritati patsientide ja kontrollisikute sülje mikrobioota võrdlemiseks bakteriaalse 16S rDNA järjestuste sekveneerimine.

Uurimistöö tulemused:

Antud doktoritöö detekteeris *STAT1* mutatsiooniga patsientidel selge interferooni signatuuri – ISG geenide ekspressioon veres oli tuntavalt tõusnud. IFN-α tase korreleerus ISG ekspressioonitasemetega hästi, samas ei olnud veres tsirkuleeriva IFN-α kontsentratsioon konstantselt kõrge. Järgnevalt uurisime kromatiini olekut patsientides. Katsetulemused näitasid, et avatud kromatiini märgis H3K4me3 oli kõrgemalt rikastunud ISG-de lähedal *STAT1* GOF patsientide genoomis. Lisaks pärast tsütokiinidega stimuleerimist nägime *STAT1* GOF rakkudes fosforüleeritud STAT1 ja STAT3 transkriptsioonifaktorite häiritud tasakaalu.

Käesolevas uurimistöös leidsime, et IL-27 mutantne p.Leu119Pro variant põhjustas märkimisväärselt madalamat *STAT1* ja *IRF1* geenide ekspressioonitaset CD4+ T rakkudes.

Antud töös detekteerisime APECED patsientide veres vähenenud MAIT rakkude arvu. Patsientide süljes esinesid IFN-α ja IL-22 vastased autoantikehad, mis omakorda võivad mõjutada suu limaskesta kaitsebarjääri funktsiooni. Suuõõne biopsia geenieksрessioonianalüüs näitas lokaalsete antimikroobsete peptiidide DEFB103A/B ja S100A12 geenieksрessiooni langust patsientides. Geenieksрessiooni andmed tõid APECED patsientides esile AHR transkriptsioonifaktori, mis on vajalik IL-22 rakkude produktsiooniks. Geeniradadeanalüüsi põhjal leidsime patsientidel kahjustunud antimikroobse vastuse ning ülemäärase põletikulise vastuse. Lisaks detekteeriti geenieksрessiooni põhjal rakkude

kahjustunud mitoos ning rakutsükkel. Samuti oli patsientide süljeproovides tõusnud proinflammatoorsete tsütokiinide tase. Lisaks sellele täheldati, et APECED patsientide sülje mikrobioota oli suurema varieeruvusega võrreldes tervete kontrollisikutega.

Uurimistöö kokkuvõte ja järeldused:

STAT1 mutatsiooniga patsientide interferooni signatuuri kinnitavad varasemad uuringud, kus *STAT1* GOF rakkude stimuleerimisel IFN-dega *in vitro* indutseeritakse märkimisväärselt kõrgemalt ISG-sid võrreldes tervete kontrollisikutega. Küsimusi tõstatab vastuolu, kuidas ISG-de kõrge tase esineb ka juhul, kui IFN-α tase on alla detektsiooni piiri. Otsesed tõendid küll veel puuduvad, kuid *STAT1* GOF patsientide hüperfosforüleeritud STAT1 ja kõrge ISG tase võib põhjustada pikemat või tugevamat seondumist kromatiinile. Sellest tulenevalt on oluline uurida kromatiini olekut patsientides. Kuna avatud kromatiini märgis on *STAT1* GOF patsientides kõrgemalt rikastunud ISG-de lähedal, võib järeldada, et muteerunud STAT1 soodustab kromatiinile seondudes epigeneetilisi muutusi, mis on vastavuses kõrgete ISG ekspressioonitasemetega ning tõusnud reaktiivsusega I tüüpi IFN-de suhtes, mis võib omakorda soodustada autoimmuunsust. Lisaks pärast tsütokiinidega stimuleerimist esineb *STAT1* GOF patsientides fosforüleeritud STAT1 ja STAT3 transkriptsioonifaktorite häiritud tasakaal, mis võib sellest tulenevalt segada STAT3 funktsioneerimist. Antud töö tulemused näitavad, et kuna viirusevastaste interferoonide toime on patsientide rakkudes võimendunud, võib see olla põhjuseks, miks neil esineb rohkem autoimmuunseid haigusi.

IL-27 mutantne p.Leu119Pro variant põhjustab märkimisväärselt madalamat *STAT1* ja *IRF1* geenide ekspressioonitaset CD4+ T rakkudes. Sellest tulenevalt võib järeldada, et IL-27 rs181206[C] variant omab kaitsvat rolli T1D vastu, kuna STAT1 signaaliraja aktiveerimine on häiritud. Antud doktoritöö tulemused ühtivad ka varem publitseeritud andmetega hiiremudelites, kus IL-27 soodustab T1D teket ning IL-27 puudulikkus inhibeerib T1D arengut.

APECED patsientide veres detekteeritud vähenenud MAIT rakkude arv võib võimendada IL-22 puudulikkust, kuna MAIT rakud on IL-22 tsütokiini produtseerijad. Patsientide süljes esinevad IFN-α ja IL-22 vastased autoantikehad võivad omakorda mõjutada suu limaskesta kaitsebarjääri funktsiooni. APECED patsientides esinevad lokaalsete antimikroobsete peptiidide DEFB103A/B ja S100A12 geeniekspressiooni langus ning häiritud AHR transkriptsioonifaktori töö, mis on vajalik IL-22 rakkude produktsiooniks. Varasemad hiireuuringud näitavad AhR inhibeerivat vastust põletikule läbi IL-22, seega APECED patsientides võib olla häiritud põletiku inhibeerimine. Patsientides on detekteeritud kahjustunud antimikroobne vastus ning võimendunud põletikuline vastus. Lisaks leiti geeniekspressiooni põhjal rakkude kahjustunud mitoos ning rakutsükkel. Sellest tulenevalt võib olla kahjustunud epiteeli kaitsefunktsioon ja paranemine. Patsientide süljes on tõusnud proinflammatoorsete tsütokiinide tase. Varasemalt on APECED patsientidel näidatud suu ja söögitoru kasvaja esinemist tingituna *Candida* infektsiooni tekitatud põletikust. Seega võib kasvaja teke olla seotud

konstantse põletiku ning IL-22 puuduliku kaitsega. APECED patsientide sülje mikrobioota suurem varieeruvus võrreldes tervete kontrollisikutega võib olla põhjustatud IL-22 defitsiidist.

Tänu monogeensete haiguste tunnustele ning geneetiliste variatsioonide esinemisele aitab käesolev doktoritöö selgitada tsütokiinide mehhanisme inimese immuunsüsteemis.

10. REFERENCES

Ahonen, P., Myllärniemi, S., Sipilä, I., & Perheentupa, J. (1990). Clinical Variation of Autoimmune Polyendocrinopathy–Candidiasis–Ectodermal Dystrophy (APECED) in a Series of 68 Patients. *New England Journal of Medicine*, *322*(26), 1829–1836. https://doi.org/10.1056/NEJM199006283222601

Al Shehri, T., Abinun, M., Gennery, A. R., Rice, G. I., Crow, Y. J., & Lilic, D. (2015). Is gain-of-function STAT1 CMC an interferonopathy? *Clinical and Experimental Immunology*, *182(S1)*(15).

Alam, M. S., Maekawa, Y., Kitamura, A., Tanigaki, K., Yoshimoto, T., Kishihara, K., & Yasutomo, K. (2010). Notch signaling drives IL-22 secretion in CD4+ T cells by stimulating the aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(13), 5943–5948. https://doi.org/10.1073/pnas.0911755107

Albanesi, C, Cavani, A., & Girolomoni, G. (1999). IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha. *Journal of Immunology (Baltimore, Md. : 1950)*, *162*(1), 494–502. http://www.ncbi.nlm.nih.gov/pubmed/9886425

Albanesi, Cristina, Scarponi, C., Cavani, A., Federici, M., Nasorri, F., & Girolomoni, G. (2000). Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-γ- and interleukin-4- induced activation of human keratinocytes. *Journal of Investigative Dermatology*, *115*(1), 81–87. https://doi.org/10.1046/j.1523-1747.2000.00041.x

Aldave, J. C., Cachay, E., Núñez, L., Chunga, A., Murillo, S., Cypowyj, S., Bustamante, J., Puel, A., Casanova, J. L., & Koo, A. (2013). A 1-year-old girl with a gain-of-function STAT1 mutation treated with hematopoietic stem cell transplantation. *Journal of Clinical Immunology*, *33*(8), 1273–1275. https://doi.org/10.1007/s10875-013-9947-5

Amatya, N., Garg, A. V., & Gaffen, S. L. (2017). IL-17 Signaling: The Yin and the Yang. In *Trends in Immunology* (Vol. 38, Issue 5, pp. 310–322). Elsevier Ltd. https://doi.org/10.1016/j.it.2017.01.006

Anderson, M. S., Venanzi, E. S., Klein, L., Chen, Z., Berzins, S. P., Turley, S. J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., & Mathis, D. (2002). Projection of an Immunological Self Shadow Within the Thymus by the Aire Protein. *Science*, *298*(5597), 1395–1401. https://doi.org/10.1126/science.1075958

Arts, R. J. W., Joosten, L. A. B., & Netea, M. G. (2018). The Potential Role of Trained Immunity in Autoimmune and Autoinflammatory Disorders. *Frontiers in Immunology*, *9*, 298. https://doi.org/10.3389/fimmu.2018.00298

Aujla, S. J., Chan, Y. R., Zheng, M., Fei, M., Askew, D. J., Pociask, D. A., Reinhart, T. A., McAllister, F., Edeal, J., Gaus, K., Husain, S., Kreindler, J. L., Dubin, P. J., Pilewski, J. M., Myerburg, M. M., Mason, C. A., Iwakura, Y., & Kolls, J. K. (2008). IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nature Medicine*, *14*(3), 275–281. https://doi.org/10.1038/nm1710

Banchereau, J., Pascual, V., & Palucka, A. K. (2004). Autoimmunity through cytokine-induced dendritic cell activation. In *Immunity* (Vol. 20, Issue 5, pp. 539–550). Cell Press. https://doi.org/10.1016/S1074-7613(04)00108-6

Barrat, F. J., Elkon, K. B., & Fitzgerald, K. A. (2016). Importance of Nucleic Acid Recognition in Inflammation and Autoimmunity. *Annual Review of Medicine*, *67*(1), 323–336. https://doi.org/10.1146/annurev-med-052814-023338

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., Plagnol, V., Pociot, F., Schuilenburg, H., Smyth, D. J., Stevens, H., Todd, J. A., Walker, N. M., Rich, S. S., Baskerville, T., … Zhang, Q. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nature Genetics*, *41*(6), 703–707. https://doi.org/10.1038/ng.381

Batten, M., Li, J., Yi, S., Kljavin, N. M., Danilenko, D. M., Lucas, S., Lee, J., de Sauvage, F. J., & Ghilardi, N. (2006). Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nature Immunology*, *7*(9), 929–936. https://doi.org/10.1038/ni1375

Båve, U., Nordmark, G., Lövgren, T., Rönnelid, J., Cajander, S., Eloranta, M.-L., Alm, G. V., & Rönnblom, L. (2005). Activation of the type I interferon system in primary Sjögren's syndrome: A possible etiopathogenic mechanism. *Arthritis & Rheumatism*, *52*(4), 1185–1195. https://doi.org/10.1002/art.20998

Bernasconi, A. R., Yancoski, J., Villa, M., Oleastro, M. M., Galicchio, M., & Rossi, J. G. (2018). Increased STAT1 Amounts Correlate with the Phospho-STAT1 Level in STAT1 Gain-of-function Defects. In *Journal of Clinical Immunology* (Vol. 38, Issue 7, pp. 745–747). Springer New York LLC. https://doi.org/10.1007/s10875-018-0557-0

Bessman, N. J., & Sonnenberg, G. F. (2016). Emerging roles for antigen presentation in establishing host-microbiome symbiosis. *Immunological Reviews*, *272*(1), 139–150. https://doi.org/10.1111/imr.12425

Betterle, C., Greggio, N. A., & Volpato, M. (1998). Autoimmune polyglandular syndrome type 1. *Journal of Clinical Endocrinology and Metabolism*, *83*(4), 1049–1055. https://doi.org/10.1210/jcem.83.4.4682

Bevins, C. L., & Salzman, N. H. (2011). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. In *Nature Reviews Microbiology* (Vol. 9, Issue 5, pp. 356–368). https://doi.org/10.1038/nrmicro2546

Bichele, R., Kärner, J., Truusalu, K., Smidt, I., Mändar, R., Conti, H. R., Gaffen, S. L., Peterson, P., Laan, M., & Kisand, K. (2018). IL-22 neutralizing autoantibodies impair fungal clearance in murine oropharyngeal candidiasis model. *European Journal of Immunology*, *48*(3), 464–470. https://doi.org/10.1002/eji.201747209

Bloomfield, M., Kanderová, V., Paračková, Z., Vrabcová, P., Svatoň, M., Froňková, E., Fejtková, M., Zachová, R., Rataj, M., Zentsová, I., Milota, T., Klocperk, A., Kalina, T., & Šedivá, A. (2018). Utility of Ruxolitinib in a Child with Chronic Mucocutaneous Candidiasis Caused by a Novel STAT1 Gain-of-Function Mutation. *Journal of Clinical Immunology*, *38*(5), 589–601. https://doi.org/10.1007/s10875-018-0519-6

Boniface, K., Bernard, F.-X., Garcia, M., Gurney, A. L., Lecron, J.-C., & Morel, F. (2005). IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes. *The Journal of Immunology*, *174*(6), 3695–3702. https://doi.org/10.4049/jimmunol.174.6.3695

Brassard, D. L., Grace, M. J., & Bordens, R. W. (2002). Interferon-alpha as an immunotherapeutic protein. *Journal of Leukocyte Biology*, *71*(4), 565–581. https://doi.org/10.1189/jlb.71.4.565

Bruserud, Ø., Bratland, E., Hellesen, A., Delaleu, N., Reikvam, H., Oftedal, B. E., & Wolff, A. S. B. (2017). Altered immune activation and IL-23 signaling in response to Candida albicans in autoimmune polyendocrine syndrome type 1. *Frontiers in Immunology*, *8*(SEP). https://doi.org/10.3389/fimmu.2017.01074

Bruserud, Ø., Siddiqui, H., Marthinussen, M. C., Chen, T., Jonsson, R., Oftedal, B. E., Olsen, I., Husebye, E. S., & Wolff, A. B. (2018). Oral microbiota in autoimmune

polyendocrine syndrome type 1. *Journal of Oral Microbiology*, *10*(1), 1442986. https://doi.org/10.1080/20002297.2018.1442986

Burbelo, P. D., Ferré, E. M. N., Chaturvedi, A., Chiorini, J. A., Alevizos, I., Lionakis, M. S., & Warner, B. M. (2019). Profiling Autoantibodies against Salivary Proteins in Sicca Conditions. *Journal of Dental Research*, *98*(7), 772–778. https://doi.org/10.1177/0022034519850564

Burgler, S., Ouaked, N., Bassin, C., Basinski, T. M., Mantel, P. Y., Siegmund, K., Meyer, N., Akdis, C. A., & Schmidt-Weber, C. B. (2009). Differentiation and functional analysis of human TH17 cells. *Journal of Allergy and Clinical Immunology*, *123*(3), 588-595.e7. https://doi.org/10.1016/j.jaci.2008.12.017

Burrack, A. L., Martinov, T., & Fife, B. T. (2017). T cell-mediated beta cell destruction: Autoimmunity and alloimmunity in the context of type 1 diabetes. In *Frontiers in Endocrinology* (Vol. 8, Issue DEC, p. 343). Frontiers Media S.A. https://doi.org/10.3389/fendo.2017.00343

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, *6*(8), 1621–1624. https://doi.org/10.1038/ismej.2012.8

Carrero, J. A., Calderon, B., Towfic, F., Artyomov, M. N., & Unanue, E. R. (2013). Defining the Transcriptional and Cellular Landscape of Type 1 Diabetes in the NOD Mouse. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0059701

Cash, H. L., Whitham, C. V., Behrendt, C. L., & Hooper, L. V. (2006). Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science*, *313*(5790), 1126–1130. https://doi.org/10.1126/science.1127119

Cella, M., Fuchs, A., Vermi, W., Facchetti, F., Otero, K., Lennerz, J. K. M., Doherty, J. M., Mills, J. C., & Colonna, M. (2009). A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*, *457*(7230), 722–725. https://doi.org/10.1038/nature07537

Chabaud, M., Garnero, P., Dayer, J. M., Guerne, P. A., Fossiez, F., & Miossec, P. (2000). Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. *Cytokine*, *12*(7), 1092–1099. https://doi.org/10.1006/cyto.2000.0681

Chen, Xiaomin, Vinkemeier, U., Zhao, Y., Jeruzalmi, D., Darnell, J. E., & Kuriyan, J. (1998). Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. *Cell*, *93*(5), 827–839. https://doi.org/10.1016/S0092-8674(00)81443-9

Chen, Xuemei, Xu, Q., Li, X., Wang, L., Yang, L., Chen, Z., Zeng, T., Xue, X., Xu, T., Wang, Y., Jia, Y., Zhao, Q., Wu, J., Liang, F., Tang, X., Yang, J., An, Y., & Zhao, X. (2020). Molecular and Phenotypic Characterization of Nine Patients with STAT1 GOF Mutations in China. *Journal of Clinical Immunology*, *40*(1), 82–95. https://doi.org/10.1007/s10875-019-00688-3

Ciecko, A. E., Foda, B., Barr, J. Y., Ramanathan, S., Atkinson, M. A., Serreze, D. V., Geurts, A. M., Lieberman, S. M., & Chen, Y. G. (2019). Interleukin-27 Is Essential for Type 1 Diabetes Development and Sjögren Syndrome-like Inflammation. *Cell Reports*, *29*(10), 3073-3086.e5. https://doi.org/10.1016/j.celrep.2019.11.010

Constantine, G. M., & Lionakis, M. S. (2019). Lessons from primary immunodeficiencies: Autoimmune regulator and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. In *Immunological Reviews* (Vol. 287, Issue 1, pp. 103–120). Blackwell Publishing Ltd. https://doi.org/10.1111/imr.12714

Costa, M. M., Saraceni, P. R., Forn-Cuní, G., Dios, S., Romero, A., Figueras, A., & Novoa, B. (2013). IL-22 is a key player in the regulation of inflammation in fish and involves innate immune cells and PI3K signaling. *Developmental & Comparative Immunology*, *41*(4), 746–755. https://doi.org/10.1016/J.DCI.2013.08.021

Crișan, T. O., Netea, M. G., & Joosten, L. A. B. (2016). Innate immune memory: Implications for host responses to damage-associated molecular patterns. *European Journal of Immunology*, *46*(4), 817–828. https://doi.org/10.1002/eji.201545497

Crow, Y. J. (2011). Type I interferonopathies: A novel set of inborn errors of immunity. *Annals of the New York Academy of Sciences*, *1238*(1), 91–98. https://doi.org/10.1111/j.1749-6632.2011.06220.x

Crow, Y. J., & Manel, N. (2015). Aicardi-Goutières syndrome and the type I interferonopathies. In *Nature Reviews Immunology* (Vol. 15, Issue 7, pp. 429–440). Nature Publishing Group. https://doi.org/10.1038/nri3850

Cucak, H., Yrlid, U., Reizis, B., Kalinke, U., & Johansson-Lindbom, B. (2009). Type I Interferon Signaling in Dendritic Cells Stimulates the Development of Lymph-Node-Resident T Follicular Helper Cells. *Immunity*, *31*(3), 491–501. https://doi.org/10.1016/j.immuni.2009.07.005

David, M. (2002). Signal transduction by type I interferons. In *BioTechniques* (Vol. 33, Issue 4 SUPPL.). Future Science Ltd London, UK . https://doi.org/10.2144/oct0207

de Beaucoudrey, L., Samarina, A., Bustamante, J., Cobat, A., Boisson-Dupuis, S., Feinberg, J., Al-Muhsen, S., Jannière, L., Rose, Y., de Suremain, M., Kong, X.-F., Filipe-Santos, O., Chapgier, A., Picard, C., Fischer, A., Dogu, F., Ikinciogullari, A., Tanir, G., Al-Hajjar, S., … Casanova, J.-L. (2010). Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine*, *89*(6), 381–402. https://doi.org/10.1097/MD.0b013e3181fdd832

Delgoffe, G. M., Murray, P. J., & Vignali, D. A. A. (2011). Interpreting mixed signals: The cell's cytokine conundrum. In *Current Opinion in Immunology* (Vol. 23, Issue 5, pp. 632–638). https://doi.org/10.1016/j.coi.2011.07.013

Delgoffe, G. M., & Vignali, D. A. A. (2013). STAT heterodimers in immunity: A mixed message or a unique signal? *JAK-STAT*, *2*(1), e23060. https://doi.org/10.4161/jkst.23060

DePaolo, R. W., Abadie, V., Tang, F., Fehlner-Peach, H., Hall, J. A., Wang, W., Marietta, E. V., Kasarda, D. D., Waldmann, T. A., Murray, J. A., Semrad, C., Kupfer, S. S., Belkaid, Y., Guandalini, S., & Jabri, B. (2011). Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature*, *471*(7337), 220–224. https://doi.org/10.1038/nature09849

Depner, M., Fuchs, S., Raabe, J., Frede, N., Glocker, C., Doffinger, R., Gkrania-Klotsas, E., Kumararatne, D., Atkinson, T. P., Schroeder, H. W., Niehues, T., Dückers, G., Stray-Pedersen, A., Baumann, U., Schmidt, R., Franco, J. L., Orrego, J., Ben-Shoshan, M., McCusker, C., … Grimbacher, B. (2016). The Extended Clinical Phenotype of 26 Patients with Chronic Mucocutaneous Candidiasis due to Gain-of-Function Mutations in STAT1. *Journal of Clinical Immunology*, *36*(1), 73–84. https://doi.org/10.1007/s10875-015-0214-9

Der, S. D., Zhou, A., Williams, B. R., & Silverman, R. H. (1998). Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(26), 15623–15628. https://doi.org/10.1073/PNAS.95.26.15623

Dhalla, F., Fox, H., Davenport, E. E., Sadler, R., Anzilotti, C., van Schouwenburg, P. A., Ferry, B., Chapel, H., Knight, J. C., & Patel, S. Y. (2016). Chronic mucocutaneous

candidiasis: characterization of a family with STAT-1 gain-of-function and development of an ex-vivo assay for Th17 deficiency of diagnostic utility. *Clinical and Experimental Immunology*, *184*(2), 216–227. https://doi.org/10.1111/cei.12746

Dhiman, R., Indramohan, M., Barnes, P. F., Nayak, R. C., Paidipally, P., Rao, L. V. M., & Vankayalapati, R. (2009). IL-22 Produced by Human NK Cells Inhibits Growth of Mycobacterium tuberculosis by Enhancing Phagolysosomal Fusion . *The Journal of Immunology*, *183*(10), 6639–6645. https://doi.org/10.4049/jimmunol.0902587

Dobeš, J., Neuwirth, A., Dobešová, M., Vobořil, M., Balounová, J., Ballek, O., Lebl, J., Meloni, A., Krohn, K., Kluger, N., Ranki, A., & Filipp, D. (2015). Gastrointestinal Autoimmunity Associated With Loss of Central Tolerance to Enteric α-Defensins. *Gastroenterology*, *149*(1), 139–150. https://doi.org/10.1053/J.GASTRO.2015.05.009

Downes, K., Pekalski, M., Angus, K. L., Hardy, M., Nutland, S., Smyth, D. J., Walker, N. M., Wallace, C., & Todd, J. A. (2010). Reduced expression of IFIH1 is protective for type 1 diabetes. *PLoS ONE*, *5*(9), 1–6. https://doi.org/10.1371/journal.pone.0012646

Dumoutier, L., Louahed, J., & Renauld, J.-C. (2000). Cloning and Characterization of IL-10-Related T Cell-Derived Inducible Factor (IL-TIF), a Novel Cytokine Structurally Related to IL-10 and Inducible by IL-9. *The Journal of Immunology*, *164*(4), 1814–1819. https://doi.org/10.4049/jimmunol.164.4.1814

Easlick, J., Szubin, R., Lantz, S., Baumgarth, N., & Abel, K. (2010). The early interferon alpha subtype response in infant macaques infected orally with SIV. *Journal of Acquired Immune Deficiency Syndromes*, *55*(1), 14–28. https://doi.org/10.1097/QAI.0b013e3181e696ca

Eidenschenk, C., Rutz, S., Liesenfeld, O., & Ouyang, W. (2014). *Role of IL-22 in Microbial Host Defense* (pp. 213–236). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-43492-5_10

Eyerich, K., Pennino, D., Scarponi, C., Foerster, S., Nasorri, F., Behrendt, H., Ring, J., Traidl-Hoffmann, C., Albanesi, C., & Cavani, A. (2009). IL-17 in atopic eczema: Linking allergen-specific adaptive and microbial-triggered innate immune response. *Journal of Allergy and Clinical Immunology*, *123*(1), 59-66.e4. https://doi.org/10.1016/j.jaci.2008.10.031

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., Cianfarani, F., Odorisio, T., Traidl-Hoffmann, C., Behrendt, H., Durham, S. R., Schmidt-Weber, C. B., & Cavani, A. (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *Journal of Clinical Investigation*, *119*(12), 3573–3585. https://doi.org/10.1172/JCI40202

Fabbri, M., Frixou, M., Degano, M., & Fousteri, G. (2019). Type 1 diabetes in STAT protein family mutations: Regulating the Th17/Treg equilibrium and beyond. *Diabetes*, *68*(2), 258–265. https://doi.org/10.2337/db18-0627

Fabris, P., Floreani, A., Tositti, G., Vergani, D., De Lalla, F., & Betterle, C. (2003). Review article: Type 1 diabetes mellitus in patients with chronic hepatitis C before and after interferon therapy. In *Alimentary Pharmacology and Therapeutics* (Vol. 18, Issue 6, pp. 549–558). https://doi.org/10.1046/j.1365-2036.2003.01681.x

Fatkhullina, A. R., Peshkova, I. O., Dzutsev, A., Aghayev, T., McCulloch, J. A., Thovarai, V., Badger, J. H., Vats, R., Sundd, P., Tang, H.-Y., Kossenkov, A. V., Hazen, S. L., Trinchieri, G., Grivennikov, S. I., & Koltsova, E. K. (2018). An Interleukin-23-Interleukin-22 Axis Regulates Intestinal Microbial Homeostasis to Protect from Diet-Induced Atherosclerosis. *Immunity*, *49*(5), 943-957.e9. https://doi.org/10.1016/j.immuni.2018.09.011

Ferre, E. M. N., Rose, S. R., Rosenzweig, S. D., Burbelo, P. D., Romito, K. R., Niemela, J. E., Rosen, L. B., Break, T. J., Gu, W., Hunsberger, S., Browne, S. K., Hsu, A. P., Rampertaap, S., Swamydas, M., Collar, A. L., Kong, H. H., Lee, C.-C. R., Chascsa, D., Simcox, T., … Lionakis, M. S. (2016). Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight*, *1*(13). https://doi.org/10.1172/JCI.INSIGHT.88782

Ferreira, R. C., Guo, H., Coulson, R. M. R., Smyth, D. J., Pekalski, M. L., Burren, O. S., Cutler, A. J., Doecke, J. D., Flint, S., McKinney, E. F., Lyons, P. A., Smith, K. G. C., Achenbach, P., Beyerlein, A., Dunger, D. B., Clayton, D. G., Wicker, L. S., Todd, J. A., Bonifacio, E., … 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

Fishman, D., Kisand, K., Hertel, C., Rothe, M., Remm, A., Pihlap, M., Adler, P., Vilo, J., Peet, A., Meloni, A., Podkrajsek, K. T., Battelino, T., Bruserud, Ø., Wolff, A. S. B., Husebye, E. S., Kluger, N., Krohn, K., Ranki, A., Peterson, H., … Peterson, P. (2017). Autoantibody Repertoire in APECED Patients Targets Two Distinct Subgroups of Proteins. *Frontiers in Immunology*, *8*, 976. https://doi.org/10.3389/fimmu.2017.00976

Forbes, L. R., Vogel, T. P., Cooper, M. A., Castro-Wagner, J., Schussler, E., Weinacht, K. G., Plant, A. S., Su, H. C., Allenspach, E. J., Slatter, M., Abinun, M., Lilic, D., Cunningham-Rundles, C., Eckstein, O., Olbrich, P., Guillerman, R. P., Patel, N. C., Demirdag, Y. Y., Zerbe, C., … Leiding, J. W. (2018). Jakinibs for the treatment of immune dysregulation in patients with gain-of-function signal transducer and activator of transcription 1 (STAT1) or STAT3 mutations. *Journal of Allergy and Clinical Immunology*, *142*(5), 1665–1669. https://doi.org/10.1016/j.jaci.2018.07.020

Fujikado, N., Mann, A. O., Bansal, K., Romito, K. R., Ferre, E. M. N., Rosenzweig, S. D., Lionakis, M. S., Benoist, C., & Mathis, D. (2016). Aire Inhibits the Generation of a Perinatal Population of Interleukin-17A-Producing γδ T Cells to Promote Immunologic Tolerance. *Immunity*, *45*(5), 999–1012. https://doi.org/10.1016/j.immuni.2016.10.023

Fujiki, R., Hijikata, A., Shirai, T., Okada, S., Kobayashi, M., & Ohara, O. (2017). Molecular mechanism and structural basis of gain-of-function of STAT1 caused by pathogenic R274Q mutation. *Journal of Biological Chemistry*, *292*(15), 6240–6254. https://doi.org/10.1074/jbc.M116.753848

Fujimoto, H., Hirase, T., Miyazaki, Y., Hara, H., Ide-Iwata, N., Nishimoto-Hazuku, A., Saris, C. J. M., Yoshida, H., & Node, K. (2011). IL-27 inhibits hyperglycemia and pancreatic islet inflammation induced by streptozotocin in mice. *American Journal of Pathology*, *179*(5), 2327–2336. https://doi.org/10.1016/j.ajpath.2011.08.001

Furumoto, Y., Smith, C. K., Blanco, L., Zhao, W., Brooks, S. R., Thacker, S. G., Zarzour, A., Sciumè, G., Tsai, W. L., Trier, A. M., Nunez, L., Mast, L., Hoffmann, V., Remaley, A. T., O'Shea, J. J., Kaplan, M. J., & Gadina, M. (2017). Tofacitinib Ameliorates Murine Lupus and Its Associated Vascular Dysfunction. *Arthritis & Rheumatology*, *69*(1), 148–160. https://doi.org/10.1002/art.39818

Gaffen, S. L. (2009). Structure and signalling in the IL-17 receptor family. In *Nature Reviews Immunology* (Vol. 9, Issue 8, pp. 556–567). https://doi.org/10.1038/nri2586

Geboes, L., Dumoutier, L., Kelchtermans, H., Schurgers, E., Mitera, T., Renauld, J.-C., & Matthys, P. (2009). Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. *Arthritis & Rheumatism*, *60*(2), 390–395. https://doi.org/10.1002/art.24220

Gibbert, K., Schlaak, J., Yang, D., & Dittmer, U. (2013). IFN-α subtypes: distinct biological activities in anti-viral therapy. *British Journal of Pharmacology*, *168*(5), 1048–1058. https://doi.org/10.1111/bph.12010

Glocker, E.-O., Hennigs, A., Nabavi, M., Schäffer, A. A., Woellner, C., Salzer, U., Pfeifer, D., Veelken, H., Warnatz, K., Tahami, F., Jamal, S., Manguiat, A., Rezaei, N., Amirzargar, A. A., Plebani, A., Hannesschläger, N., Gross, O., Ruland, J., & Grimbacher, B. (2009). A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *The New England Journal of Medicine*, *361*(18), 1727–1735. https://doi.org/10.1056/NEJMoa0810719

Goepfert, A., Lehmann, S., Blank, J., Kolbinger, F., & Rondeau, J. M. (2020). Structural Analysis Reveals that the Cytokine IL-17F Forms a Homodimeric Complex with Receptor IL-17RC to Drive IL-17RA-Independent Signaling. *Immunity*, *52*(3), 499-512.e5. https://doi.org/10.1016/j.immuni.2020.02.004

Green, D. S., Young, H. A., & Valencia, J. C. (2017). Current prospects of type II interferon signaling and autoimmunity Biological role of IFN on inflammation. *J Biol Chem*, *292*(34), 13925–13933. https://doi.org/10.1074/jbc.R116.774745

Gronke, K., Hernández, P. P., Zimmermann, J., Klose, C. S. N., Kofoed-Branzk, M., Guendel, F., Witkowski, M., Tizian, C., Amann, L., Schumacher, F., Glatt, H., Triantafyllopoulou, A., & Diefenbach, A. (2019). Interleukin-22 protects intestinal stem cells against genotoxic stress. *Nature*, *566*(7743), 249–253. https://doi.org/10.1038/s41586-019-0899-7

Gu, C., Wu, L., & Li, X. (2013). IL-17 family: Cytokines, receptors and signaling. In *Cytokine* (Vol. 64, Issue 2, pp. 477–485). https://doi.org/10.1016/j.cyto.2013.07.022

Halonen, M., Eskelin, P., Myhre, A.-G., Perheentupa, J., Husebye, E. S., Kämpe, O., Rorsman, F., Peltonen, L., Ulmanen, I., & Partanen, J. (2002). AIRE Mutations and Human Leukocyte Antigen Genotypes as Determinants of the Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Phenotype. *The Journal of Clinical Endocrinology & Metabolism*, *87*(6), 2568–2574. https://doi.org/10.1210/jcem.87.6.8564

Harrison, C., Kiladjian, J.-J., Al-Ali, H. K., Gisslinger, H., Waltzman, R., Stalbovskaya, V., McQuitty, M., Hunter, D. S., Levy, R., Knoops, L., Cervantes, F., Vannucchi, A. M., Barbui, T., & Barosi, G. (2012). JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. *New England Journal of Medicine*, *366*(9), 787–798. https://doi.org/10.1056/NEJMoa1110556

Hasegawa, M., Yada, S., Liu, M. Z., Kamada, N., Muñoz-Planillo, R., Do, N., Núñez, G., & Inohara, N. (2014). Interleukin-22 Regulates the Complement System to Promote Resistance against Pathobionts after Pathogen-Induced Intestinal Damage. *Immunity*, *41*(4), 620–632. https://doi.org/10.1016/j.immuni.2014.09.010

Heino, M., Peterson, P., Kudoh, J., Nagamine, K., Lagerstedt, A., Ovod, V., Ranki, A., Rantala, I., Nieminen, M., Tuukkanen, J., Scott, H. S., Antonarakis, S. E., Shimizu, N., & Krohn, K. (1999). Autoimmune Regulator Is Expressed in the Cells Regulating Immune Tolerance in Thymus Medulla. *Biochemical and Biophysical Research Communications*, *257*(3), 821–825. https://doi.org/10.1006/bbrc.1999.0308

Hetemäki, I., Jarva, H., Kluger, N., Baldauf, H.-M., Laakso, S., Bratland, E., Husebye, E. S., Kisand, K., Ranki, A., Peterson, P., & Arstila, T. P. (2016). Anticommensal Responses Are Associated with Regulatory T Cell Defect in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Patients. *Journal of Immunology (Baltimore, Md. : 1950)*, *196*(7), 2955–2964. https://doi.org/10.4049/jimmunol.1500301

Higgins, E., Al Shehri, T., McAleer, M. A., Conlon, N., Feighery, C., Lilic, D., & Irvine, A. D. (2015). Use of ruxolitinib to successfully treat chronic mucocutaneous candidiasis caused by gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation. *Journal of Allergy and Clinical Immunology*, *135*(2), 551-553.e3. https://doi.org/10.1016/J.JACI.2014.12.1867

Hiller, J., Hagl, B., Effner, R., Puel, A., Schaller, M., Mascher, B., Eyerich, S., Eyerich, K., Jansson, A. F., Ring, J., Casanova, J. L., Renner, E. D., & Traidl-Hoffmann, C. (2018). STAT1 Gain-of-Function and Dominant Negative STAT3 Mutations Impair IL-17 and IL-22 Immunity Associated with CMC. *Journal of Investigative Dermatology*, *138*(3), 711–714. https://doi.org/10.1016/j.jid.2017.09.035

Hoffmann, H.-H., Schneider, W. M., & Rice, C. M. (2015). Interferons and viruses: an evolutionary arms race of molecular interactions. *Trends in Immunology*, *36*(3), 124–138. https://doi.org/10.1016/J.IT.2015.01.004

Horvath, C. M., Stark, G. R., Kerr, I. M., & Darnell, J. E. (1996). Interactions between STAT and non-STAT proteins in the interferon-stimulated gene factor 3 transcription complex. *Molecular and Cellular Biology*, *16*(12), 6957–6964. https://doi.org/10.1128/mcb.16.12.6957

Hosking, L. M., Quach, A., Slade, C. A., Galea, M. A., Richards, S., Choo, S., & Ferrante, A. (2020). Proceed with Caution: STAT1 GOF Diagnosis Missed Due to Intronic SNP. In *Journal of Clinical Immunology* (Vol. 40, Issue 3, pp. 547–550). Springer. https://doi.org/10.1007/s10875-020-00768-9

Hu, X., & Ivashkiv, L. B. (2009). Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. *Immunity*, *31*(4), 539–550. https://doi.org/10.1016/j.immuni.2009.09.002

Huang, X., Yuan, J., Goddard, A., Foulis, A., James, R. F. L., Lernmark, Å., Pujol-Borrell, R., Rabinovitch, A., Somoza, N., & Stewart, T. A. (1995). Interferon expression in the pancreases of patients with type I diabetes. *Diabetes*, *44*(6), 658–664. https://doi.org/10.2337/diab.44.6.658

Husebye, E S, Perheentupa, J., Rautemaa, R., & Kämpe, O. (2009). Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *Journal of Internal Medicine*, *265*(5), 514–529. https://doi.org/10.1111/j.1365-2796.2009.02090.x

Husebye, Eystein S., & Anderson, M. S. (2010). Autoimmune Polyendocrine Syndromes: Clues to Type 1 Diabetes Pathogenesis. *Immunity*, *32*(4), 479–487. https://doi.org/10.1016/J.IMMUNI.2010.03.016

Ivanov, I. I., Frutos, R. de L., Manel, N., Yoshinaga, K., Rifkin, D. B., Sartor, R. B., Finlay, B. B., & Littman, D. R. (2008). Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. *Cell Host and Microbe*, *4*(4), 337–349. https://doi.org/10.1016/j.chom.2008.09.009

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. 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

Ivashkiv, L. B. (2018). IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. In *Nature Reviews Immunology* (Vol. 18, Issue 9, pp. 545–558). Nature Publishing Group. https://doi.org/10.1038/s41577-018-0029-z

Jean-Baptiste, V. S. E., Xia, C. Q., Clare-Salzler, M. J., & Horwitz, M. S. (2017). Type 1 Diabetes and Type 1 Interferonopathies: Localization of a Type 1 Common Thread of

Virus Infection in the Pancreas. In *EBioMedicine* (Vol. 22, pp. 10–17). Elsevier B.V. https://doi.org/10.1016/j.ebiom.2017.06.014

Kamada, R., Yang, W., Zhang, Y., Patel, M. C., Yang, Y., Ouda, R., Dey, A., Wakabayashi, Y., Sakaguchi, K., Fujita, T., Tamura, T., Zhu, J., & Ozato, K. (2018). Interferon stimulation creates chromatin marks and establishes transcriptional memory. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(39), E9162–E9171. https://doi.org/10.1073/pnas.1720930115

Kärner, J, Meager, A., Laan, M., Maslovskaja, J., Pihlap, M., Remm, A., Juronen, E., Wolff, A. S. B., Husebye, E. S., Podkrajšek, K. T., Bratanic, N., Battelino, T., Willcox, N., Peterson, P., & Kisand, K. (2013). Anti-cytokine autoantibodies suggest pathogenetic links with autoimmune regulator deficiency in humans and mice. *Clinical and Experimental Immunology*, *171*(3), 263–272. https://doi.org/10.1111/cei.12024

Kärner, Jaanika, Pihlap, M., Ranki, A., Krohn, K., Trebusak Podkrajsek, K., Bratanic, N., Battelino, T., Willcox, N., Peterson, P., & Kisand, K. (2016). IL-6-specific autoantibodies among APECED and thymoma patients. *Immunity, Inflammation and Disease*, *4*(2), 235–243. https://doi.org/10.1002/iid3.109

Kasela, S., Kisand, K., Tserel, L., Kaleviste, E., Remm, A., Fischer, K., Esko, T., Westra, H. J., Fairfax, B. P., Makino, S., Knight, J. C., Franke, L., Metspalu, A., Peterson, P., & Milani, L. (2017). Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4 + versus CD8 + T cells. *PLoS Genetics*, *13*(3), e1006643. https://doi.org/10.1371/journal.pgen.1006643

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. In *Nature Immunology* (Vol. 11, Issue 5, pp. 373–384). Nature Publishing Group. https://doi.org/10.1038/ni.1863

Kawasaki, T., Kawai, T., & Akira, S. (2011). Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. In *Immunological Reviews* (Vol. 243, Issue 1, pp. 61–73). https://doi.org/10.1111/j.1600-065X.2011.01048.x

Kisand, K., Link, M., Wolff, A. S. B., Meager, A., Tserel, L., Org, T., Murumagi, A., Uibo, R., Willcox, N., Trebusak Podkrajsek, K., Battelino, T., Lobell, A., Kampe, O., Lima, K., Meloni, A., Ergun-Longmire, B., Maclaren, N. K., Perheentupa, J., Krohn, K. J. E., … Peterson, P. (2008). Interferon autoantibodies associated with AIRE deficiency decrease the expression of IFN-stimulated genes. *Blood*, *112*(7), 2657–2666. https://doi.org/10.1182/blood-2008-03-144634

Kisand, Kai, Bøe Wolff, A. S., Podkrajsek, K. T., Tserel, L., Link, M., Kisand, K. V, Ersvaer, E., Perheentupa, J., Erichsen, M. M., Bratanic, N., Meloni, A., Cetani, F., Perniola, R., Ergun-Longmire, B., Maclaren, N., Krohn, K. J. E., Pura, M., Schalke, B., Ströbel, P., … Meager, A. (2010). Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *The Journal of Experimental Medicine*, *207*(2), 299–308. https://doi.org/10.1084/jem.20091669

Kisand, Kai, Lilic, D., Casanova, J.-L., Peterson, P., Meager, A., & Willcox, N. (2011). Mucocutaneous candidiasis and autoimmunity against cytokines in APECED and thymoma patients: Clinical and pathogenetic implications. *European Journal of Immunology*, *41*(6), 1517–1527. https://doi.org/10.1002/eji.201041253

Kisand, Kai, & Peterson, P. (2015). Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy. *Journal of Clinical Immunology*, *35*(5), 463–478. https://doi.org/10.1007/s10875-015-0176-y

Kiykim, A., Charbonnier, L. M., Akcay, A., Karakoc-Aydiner, E., Ozen, A., Ozturk, G., Chatila, T. A., & Baris, S. (2019). Hematopoietic Stem Cell Transplantation in Patients with Heterozygous STAT1 Gain-of-Function Mutation. *Journal of Clinical Immunology*, *39*(1), 37–44. https://doi.org/10.1007/s10875-018-0575-y

Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V. K. (2009). IL-17 and Th17 Cells. *Annual Review of Immunology*, *27*(1), 485–517. https://doi.org/10.1146/annurev.immunol.021908.132710

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M. T., Martin, T. J., & Suda, T. (1999). IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *Journal of Clinical Investigation*, *103*(9), 1345–1352. https://doi.org/10.1172/JCI5703

Kotenko, S. V., Gallagher, G., Baurin, V. V., Lewis-Antes, A., Shen, M., Shah, N. K., Langer, J. A., Sheikh, F., Dickensheets, H., & Donnelly, R. P. (2003). IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature Immunology*, *4*(1), 69–77. https://doi.org/10.1038/ni875

Kryczek, I., Wei, S., Zou, L., Altuwaijri, S., Szeliga, W., Kolls, J., Chang, A., & Zou, W. (2007). Cutting Edge: Th17 and Regulatory T Cell Dynamics and the Regulation by IL-2 in the Tumor Microenvironment. *The Journal of Immunology*, *178*(11), 6730–6733. https://doi.org/10.4049/jimmunol.178.11.6730

Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., Kheradpour, P., Zhang, Z., Wang, J., Ziller, M. J., Amin, V., Whitaker, J. W., Schultz, M. D., Ward, L. D., Sarkar, A., Quon, G., Sandstrom, R. S., Eaton, M. L., Wu, Y. C., … Kellis, M. (2015). Integrative analysis of 111 reference human epigenomes. *Nature*, *518*(7539), 317–330. https://doi.org/10.1038/nature14248

Laakso, S. M., Kekäläinen, E., Heikkilä, N., Mannerström, H., Kisand, K., Peterson, P., Ranki, A., & Arstila, T. P. (2014). *In vivo* analysis of helper T cell responses in patients with autoimmune polyendocrinopathy – candidiasis – ectodermal dystrophy provides evidence in support of an IL-22 defect. *Autoimmunity*, *47*(8), 556–562. https://doi.org/10.3109/08916934.2014.929666

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Lee, Y., Awasthi, A., Yosef, N., Quintana, F. J., Xiao, S., Peters, A., Wu, C., Kleinewietfeld, M., Kunder, S., Hafler, D. A., Sobel, R. A., Regev, A., & Kuchroo, V. K. (2012). Induction and molecular signature of pathogenic T H 17 cells. *Nature Immunology*, *13*(10), 991–999. https://doi.org/10.1038/ni.2416

Leeansyah, E., Loh, L., Nixon, D. F., & Sandberg, J. K. (2014). Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nature Communications*, *5*(1), 3143. https://doi.org/10.1038/ncomms4143

Legoux, F., Bellet, D., Daviaud, C., El Morr, Y., Darbois, A., Niort, K., Procopio, E., Salou, M., Gilet, J., Ryffel, B., Balvay, A., Foussier, A., Sarkis, M., El Marjou, A., Schmidt, F., Rabot, S., & Lantz, O. (2019). Microbial metabolites control the thymic development of mucosal-associated invariant T cells. *Science*, *366*(6464), 494–499. https://doi.org/10.1126/science.aaw2719

LeibundGut-Landmann, S., Groß, O., Robinson, M. J., Osorio, F., Slack, E. C., Tsoni, S. V., Schweighoffer, E., Tybulewicz, V., Brown, G. D., Ruland, J., & Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the

induction of T helper cells that produce interleukin 17. *Nature Immunology*, *8*(6), 630–638. https://doi.org/10.1038/ni1460

Leiding, J. W., Okada, S., Hagin, D., Abinun, M., Shcherbina, A., Balashov, D. N., Kim, V. H. D., Ovadia, A., Guthery, S. L., Pulsipher, M., Lilic, D., Devlin, L. A., Christie, S., Depner, M., Fuchs, S., van Royen-Kerkhof, A., Lindemans, C., Petrovic, A., Sullivan, K. E., … Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation and the Primary Immune Deficiency Treatment Consortium. (2018). Hematopoietic stem cell transplantation in patients with gain-of-function signal transducer and activator of transcription 1 mutations. *Journal of Allergy and Clinical Immunology*, *141*(2), 704-717.e5. https://doi.org/10.1016/j.jaci.2017.03.049

Lejeune, D., Dumoutier, L., Constantinescu, S., Kruijer, W., Schuringa, J. J., & Renauld, J. C. (2002). Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line: Pathways that are shared with and distinct from IL-10. *Journal of Biological Chemistry*, *277*(37), 33676–33682. https://doi.org/10.1074/jbc.M204204200

Levy, D. E., & Darnell, J. E. (2002). Stats: transcriptional control and biological impact. *Nature Reviews. Molecular Cell Biology*, *3*(9), 651–662. https://doi.org/10.1038/nrm909

Li, Q., Xu, B., Michie, S. A., Rubins, K. H., Schreriber, R. D., & McDevitt, H. O. (2008). Interferon-α initiates type 1 diabetes in nonobese diabetic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(34), 12439–12444. https://doi.org/10.1073/pnas.0806439105

Liang, S. C., Nickerson-Nutter, C., Pittman, D. D., Carrier, Y., Goodwin, D. G., Shields, K. M., Lambert, A.-J., Schelling, S. H., Medley, Q. G., Ma, H.-L., Collins, M., Dunussi-Joannopoulos, K., & Fouser, L. A. (2010). IL-22 Induces an Acute-Phase Response. *The Journal of Immunology*, *185*(9), 5531–5538. https://doi.org/10.4049/jimmunol.0904091

Liang, S. C., Tan, X.-Y., Luxenberg, D. P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., & Fouser, L. A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *The Journal of Experimental Medicine*, *203*(10), 2271–2279. https://doi.org/10.1084/jem.20061308

Liang, Y., Xu, W. D., Yang, X. K., Fang, X. Y., Liu, Y. Y., Ni, J., Qiu, L. J., Hui, P., Cen, H., Leng, R. X., Pan, H. F., & Ye, D. Q. (2014). Association of signaling transducers and activators of transcription 1 and systemic lupus erythematosus. In *Autoimmunity* (Vol. 47, Issue 3, pp. 141–145). Informa Healthcare. https://doi.org/10.3109/08916934.2013.873415

Liau, N. P. D., Laktyushin, A., Lucet, I. S., Murphy, J. M., Yao, S., Whitlock, E., Callaghan, K., Nicola, N. A., Kershaw, N. J., & Babon, J. J. (2018). The molecular basis of JAK/STAT inhibition by SOCS1. *Nature Communications*, *9*(1), 1–14. https://doi.org/10.1038/s41467-018-04013-1

Lindh, E., Rosmaraki, E., Berg, L., Brauner, H., Karlsson, M. C. I., Peltonen, L., Höglund, P., & Winqvist, O. (2010). AIRE deficiency leads to impaired iNKT cell development. *Journal of Autoimmunity*, *34*(1), 66–72. https://doi.org/10.1016/j.jaut.2009.07.002

Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D., & Shuai, K. E. (1998). Inhibition of Stat1-mediated gene activation by PIAS1. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(18), 10626–10631. https://doi.org/10.1073/pnas.95.18.10626

Liu, L., Okada, S., Kong, X.-F., Kreins, A. Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y., Audry, M., Nitschke, P., Masson, C., Toth, B., Flatot, J., Migaud, M., Chrabieh, M., Kochetkov, T., Bolze, A., Borghesi, A., Toulon, A., … Casanova, J.-L. (2011). Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *The Journal of Experimental Medicine*, *208*(8), 1635–1648. https://doi.org/10.1084/jem.20110958

Lorenzini, T., Dotta, L., Giacomelli, M., Vairo, D., & Badolato, R. (2017). *Review STAT mutations as program switchers : turning primary immunodeficiencies into autoimmune diseases*. *101*(January), 29–38. https://doi.org/10.1189/jlb.5RI0516-237RR

Lubberts, E., Koenders, M. I., Oppers-Walgreen, B., Van Den Bersselaar, L., Coenen-De Roo, C. J. J., Joosten, L. A. B., & Van Den Berg, W. B. (2004). Treatment with a Neutralizing Anti-Murine Interleukin-17 Antibody after the Onset of Collagen-Induced Arthritis Reduces Joint Inflammation, Cartilage Destruction, and Bone Erosion. *Arthritis and Rheumatism*, *50*(2), 650–659. https://doi.org/10.1002/art.20001

Lucas, S., Ghilardi, N., Li, J., & De Sauvage, F. J. (2003). IL-27 regulates IL-12 responsiveness of naïve CD4+ T cells through Stat1-dependent and -independent mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(25), 15047–15052. https://doi.org/10.1073/pnas.2536517100

Mandal, A., & Viswanathan, C. (2015). Natural killer cells: In health and disease. In *Hematology/ Oncology and Stem Cell Therapy* (Vol. 8, Issue 2, pp. 47–55). King Faisal Specialist Hospital and Research Centre. https://doi.org/10.1016/j.hemonc.2014.11.006

Mann, E. R., Bernardo, D., Ng, S. C., Rigby, R. J., Al-Hassi, H. O., Landy, J., Peake, S. T. C., Spranger, H., English, N. R., Thomas, L. V., Stagg, A. J., Knight, S. C., & Hart, A. L. (2014). Human Gut Dendritic Cells Drive Aberrant Gut-specific T-cell Responses in Ulcerative Colitis, Characterized by Increased IL-4 Production and Loss of IL-22 and IFNγ. *Inflammatory Bowel Diseases*, *20*(12), 2299–2307. https://doi.org/10.1097/MIB.0000000000000223

Marroqui, L., Dos Santos, R. S., Op de beeck, A., Coomans de Brachène, A., Marselli, L., Marchetti, P., & Eizirik, D. L. (2017). Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia*, *60*(4), 656–667. https://doi.org/10.1007/s00125-016-4201-3

Martinez-Martinez, L., Martinez-Saavedra, M. T., Fuentes-Prior, P., Barnadas, M., Rubiales, M. V., Noda, J., Badell, I., Rodríguez-Gallego, C., & Calle-Martin, O. de la. (2015). A novel gain-of-function STAT1 mutation resulting in basal phosphorylation of STAT1 and increased distal IFN-γ-mediated responses in chronic mucocutaneous candidiasis. *Molecular Immunology*, *68*(2 Pt C), 597–605. https://doi.org/10.1016/j.molimm.2015.09.014

McLean, C. Y., Bristor, D., Hiller, M., Clarke, S. L., Schaar, B. T., Lowe, C. B., Wenger, A. M., & Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. *Nature Biotechnology*, *28*(5), 495–501. https://doi.org/10.1038/nbt.1630

Meager, A., Wadhwa, M., Dilger, P., Bird, C., Thorpe, R., Newsom-Davis, J., & Willcox, N. (2003). Anti-cytokine autoantibodies in autoimmunity: Preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. *Clinical and Experimental Immunology*, *132*(1), 128–136. https://doi.org/10.1046/j.1365-2249.2003.02113.x

Meager, Anthony, Visvalingam, K., Peterson, P., Möll, K., Murumägi, A., Krohn, K., Eskelin, P., Perheentupa, J., Husebye, E., Kadota, Y., & Willcox, N. (2006). Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Medicine*, *3*(7), 1152–1164. https://doi.org/10.1371/journal.pmed.0030289

Meesilpavikkai, K., Dik, W. A., Schrijver, B., Nagtzaam, N. M. A., van Rijswijk, A., Driessen, G. J., van der Spek, P. J., van Hagen, P. M., & Dalm, V. A. S. H. (2017). A novel heterozygous mutation in the STAT1 SH2 domain causes chronic mucocutaneous candidiasis, atypically diverse infections, autoimmunity, and impaired cytokine regulation. *Frontiers in Immunology*, *8*(MAR). https://doi.org/10.3389/fimmu.2017.00274

Meka, R. R., Venkatesha, S. H., Dudics, S., Acharya, B., & Moudgil, K. D. (2015). IL-27-induced modulation of autoimmunity and its therapeutic potential. In *Autoimmunity Reviews* (Vol. 14, Issue 12, pp. 1131–1141). Elsevier B.V. https://doi.org/10.1016/j.autrev.2015.08.001

Mertens, C., Zhong, M., Krishnaraj, R., Zou, W., Chen, X., Darnell, J. E., & Jr. (2006). Dephosphorylation of phosphotyrosine on STAT1 dimers requires extensive spatial reorientation of the monomers facilitated by the N-terminal domain. *Genes & Development*, *20*(24), 3372–3381. https://doi.org/10.1101/gad.1485406

Meyer, S., Woodward, M., Hertel, C., Vlaicu, P., Haque, Y., Kärner, J., Macagno, A., Onuoha, S. C., Fishman, D., Peterson, H., Metsküla, K., Uibo, R., Jäntti, K., Hokynar, K., Wolff, A. S. B., APECED patient collaborative, A. patient, Krohn, K., Ranki, A., Peterson, P., … Hayday, A. (2016). AIRE-Deficient Patients Harbor Unique High-Affinity Disease-Ameliorating Autoantibodies. *Cell*, *166*(3), 582–595. https://doi.org/10.1016/j.cell.2016.06.024

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., Kawamura, N., Ariga, T., Pasic, S., Stojkovic, O., Metin, A., & 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

Miyazaki, Y., Inoue, H., Matsumura, M., Matsumoto, K., Nakano, T., Tsuda, M., Hamano, S., Yoshimura, A., & Yoshida, H. (2005). Exacerbation of Experimental Allergic Asthma by Augmented Th2 Responses in WSX-1-Deficient Mice. *The Journal of Immunology*, *175*(4), 2401–2407. https://doi.org/10.4049/jimmunol.175.4.2401

Miyazaki, Y., Shimanoe, Y., Wang, S., & Yoshida, H. (2008). Amelioration of delayed-type hypersensitivity responses by IL-27 administration. *Biochemical and Biophysical Research Communications*, *373*(3), 397–402. https://doi.org/10.1016/j.bbrc.2008.06.038

Mizoguchi, Y., Tsumura, M., Okada, S., Hirata, O., Minegishi, S., Imai, K., Hyakuna, N., Muramatsu, H., Kojima, S., Ozaki, Y., Imai, T., Takeda, S., Okazaki, T., Ito, T., Yasunaga, S., Takihara, Y., Bryant, V. L., Kong, X.-F., Cypowyj, S., … Kobayashi, M. (2014). Simple diagnosis of STAT1 gain-of-function alleles in patients with chronic mucocutaneous candidiasis. *Journal of Leukocyte Biology*, *95*(4), 667–676. https://doi.org/10.1189/jlb.0513250

Moll, H. P., Maier, T., Zommer, A., Lavoie, T., & Brostjan, C. (2011). The differential activity of interferon-α subtypes is consistent among distinct target genes and cell types. *Cytokine*, *53*(1), 52–59. https://doi.org/10.1016/j.cyto.2010.09.006

Monteleone, I., Rizzo, A., Sarra, M., Sica, G., Sileri, P., Biancone, L., MacDonald, T. T., Pallone, F., & Monteleone, G. (2011). Aryl Hydrocarbon Receptor-Induced Signals

Up-regulate IL-22 Production and Inhibit Inflammation in the Gastrointestinal Tract. *Gastroenterology*, *141*(1), 237-248.e1. https://doi.org/10.1053/j.gastro.2011.04.007

Moriya, K., Suzuki, T., Uchida, N., Nakano, T., Katayama, S., Irie, M., Rikiishi, T., Niizuma, H., Okada, S., Imai, K., Sasahara, Y., & Kure, S. (2020). Ruxolitinib treatment of a patient with steroid-dependent severe autoimmunity due to STAT1 gain-of-function mutation. *International Journal of Hematology*, 1–5. https://doi.org/10.1007/s12185-020-02860-7

Mössner, R., Diering, N., Bader, O., Forkel, S., Overbeck, T., Gross, U., Grimbacher, B., Schön, M. P., & Buhl, T. (2016). Ruxolitinib Induces Interleukin 17 and Ameliorates Chronic Mucocutaneous Candidiasis Caused by STAT1 Gain-of-Function Mutation. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *62*(7), 951–953. https://doi.org/10.1093/cid/ciw020

Muñoz, M., Eidenschenk, C., Ota, N., Wong, K., Lohmann, U., Kühl, A. A., Wang, X., Manzanillo, P., Li, Y., Rutz, S., Zheng, Y., Diehl, L., Kayagaki, N., van Lookeren-Campagne, M., Liesenfeld, O., Heimesaat, M., & Ouyang, W. (2015). Interleukin-22 Induces Interleukin-18 Expression from Epithelial Cells during Intestinal Infection. *Immunity*, *42*(2), 321–331. https://doi.org/10.1016/j.immuni.2015.01.011

Myhre, A. G., Halonen, M., Eskelin, P., Ekwall, O., Hedstrand, H., Rorsman, F., Kampe, O., & Husebye, E. S. (2001). Autoimmune polyendocrine syndrome type 1 (APS I) in Norway. *Clinical Endocrinology*, *54*(2), 211–217. https://doi.org/10.1046/j.1365-2265.2001.01201.x

Nagamine, K., Peterson, P., Scott, H. S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K. J. E., Lalioti, M. D., Mullis, P. E., Antonarakis, S. E., Kawasaki, K., Asakawa, S., Ito, F., & Shimizu, N. (1997). Positional cloning of the APECED gene. *Nature Genetics*, *17*(4), 393–398. https://doi.org/10.1038/ng1297-393

Napetschnig, J., & Wu, H. (2013). Molecular Basis of NF-κB Signaling. *Annual Review of Biophysics*, *42*(1), 443–468. https://doi.org/10.1146/annurev-biophys-083012-130338

Neil, J. A., Matsuzawa-Ishimoto, Y., Kernbauer-Hölzl, E., Schuster, S. L., Sota, S., Venzon, M., Dallari, S., Galvao Neto, A., Hine, A., Hudesman, D., Loke, P., Nice, T. J., & Cadwell, K. (2019). IFN-I and IL-22 mediate protective effects of intestinal viral infection. *Nature Microbiology*, *4*(10), 1737–1749. https://doi.org/10.1038/s41564-019-0470-1

Ness-Schwickerath, K. J., & Morita, C. T. (2011). Regulation and function of IL-17A- and IL-22-producing γδ T cells. *Cellular and Molecular Life Sciences*, *68*(14), 2371–2390. https://doi.org/10.1007/s00018-011-0700-z

Netea, M. G., Joosten, L. A. B., Latz, E., Mills, K. H. G., Natoli, G., Stunnenberg, H. G., ONeill, L. A. J., & Xavier, R. J. (2016). Trained immunity: A program of innate immune memory in health and disease. *Science*, *352*(6284), aaf1098–aaf1098. https://doi.org/10.1126/science.aaf1098

Neufert, C., Becker, C., Wirtz, S., Fantini, M. C., Weigmann, B., Galle, P. R., & Neurath, M. F. (2007). IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on STAT1. *European Journal of Immunology*, *37*(7), 1809–1816. https://doi.org/10.1002/eji.200636896

Ng, C. T., Mendoza, J. L., Garcia, K. C., & Oldstone, M. B. A. (2016). Alpha and Beta Type 1 Interferon Signaling: Passage for Diverse Biologic Outcomes. In *Cell* (Vol. 164, Issue 3, pp. 349–352). Cell Press. https://doi.org/10.1016/j.cell.2015.12.027

Ng, W.-F., von Delwig, A., Carmichael, A. J., Arkwright, P. D., Abinun, M., Cant, A. J., Jolles, S., & Lilic, D. (2010). Impaired TH17 responses in patients with chronic

mucocutaneous candidiasis with and without autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy. *Journal of Allergy and Clinical Immunology*, *126*(5), 1006-1015.e4. https://doi.org/10.1016/j.jaci.2010.08.027

Nielsen, J., Kofod-Olsen, E., Spaun, E., Larsen, C. S., Christiansen, M., & Mogensen, T. H. (2015). A STAT1-gain-of-function mutation causing Th17 deficiency with chronic mucocutaneous candidiasis, psoriasiform hyperkeratosis and dermatophytosis. *BMJ Case Reports*, *2015*. https://doi.org/10.1136/bcr-2015-211372

Nograles, K. E., Zaba, L. C., Guttman-Yassky, E., Fuentes-Duculan, J., Suárez-Fariñas, M., Cardinale, I., Khatcherian, A., Gonzalez, J., Pierson, K. C., White, T. R., Pensabene, C., Coats, I., Novitskaya, I., Lowes, M. A., & Krueger, J. G. (2008). Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *British Journal of Dermatology*, *159*(5), 1092–1102. https://doi.org/10.1111/j.1365-2133.2008.08769.x

Norian, L. A., Rodriguez, P. C., O'Mara, L. A., Zabaleta, J., Ochoa, A. C., Cella, M., & Allen, P. M. (2009). Tumor-Infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-Arginine metabolism. *Cancer Research*, *69*(7), 3086–3094. https://doi.org/10.1158/0008-5472.CAN-08-2826

Nurieva, R. I., Chung, Y., Hwang, D., Yang, X. O., Kang, H. S., Ma, L., Wang, Y. hong, Watowich, S. S., Jetten, A. M., Tian, Q., & Dong, C. (2008). Generation of T Follicular Helper Cells Is Mediated by Interleukin-21 but Independent of T Helper 1, 2, or 17 Cell Lineages. *Immunity*, *29*(1), 138–149. https://doi.org/10.1016/j.immuni.2008.05.009

Oftedal, B. E., Marthinussen, M. C., Erichsen, M. M., Tveitarås, M. K., Kjellesvik-Kristiansen, A., Hammenfors, D., Jonsson, M. V., Kisand, K., Jonsson, R., & Wolff, A. S. B. (2017). Impaired salivary gland activity in patients with autoimmune poly-endocrine syndrome type I. *Autoimmunity*, *50*(4), 211–222. https://doi.org/10.1080/08916934.2017.1344972

Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M., Nishihara, M., Iwakura, Y., & Hirano, T. (2008). Interleukin-17 Promotes Auto-immunity by Triggering a Positive-Feedback Loop via Interleukin-6 Induction. *Immunity*, *29*(4), 628–636. https://doi.org/10.1016/j.immuni.2008.07.018

Oka, R., Hiroi, N., Shigemitsu, R., Sue, M., Oshima, Y., & Yoshida-Hiroi, M. (2011). Type 1 diabetes mellitus associated with pegylated interferon-α plus ribavirin treat-ment for chronic hepatitis C: Case report and literature review. In *Clinical Medicine Insights: Endocrinology and Diabetes* (Vol. 4, pp. 39–45). Libertas Academica Ltd. https://doi.org/10.4137/CMED.S7815

Onishi, R. M., & Gaffen, S. L. (2010). Interleukin-17 and its target genes: Mechanisms of interleukin-17 function in disease. In *Immunology* (Vol. 129, Issue 3, pp. 311–321). https://doi.org/10.1111/j.1365-2567.2009.03240.x

Ortega, C., Fernández-A, S., Carrillo, J. M., Romero, P., Molina, I. J., Moreno, J. C., & Santamaría, M. (2009). IL-17-producing CD8 + T lymphocytes from psoriasis skin plaques are cytotoxic effector cells that secrete Th17-related cytokines . *Journal of Leukocyte Biology*, *86*(2), 435–443. https://doi.org/10.1189/jlb.0109046

Paget, C., Ivanov, S., Fontaine, J., Renneson, J., Blanc, F., Pichavant, M., Dumoutier, L., Ryffel, B., Renauld, J. C., Gosset, P., Gosset, P., Si-Tahar, M., Faveeuw, C., & Trottein, F. (2012). Interleukin-22 Is Produced by Invariant Natural Killer T Lymphocytes during Influenza A Virus Infection. *Journal of Biological Chemistry*, *287*(12), 8816–8829. https://doi.org/10.1074/jbc.M111.304758

Pandya, A. D., Al-Jaderi, Z., Høglund, R. A., Holmøy, T., Harbo, H. F., Norgauer, J., & Maghazachi, A. A. (2011). Identification of human NK17/NK1 cells. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0026780

Park, S. H., Kang, K., Giannopoulou, E., Qiao, Y., Kang, K., Kim, G., Park-Min, K. H., & Ivashkiv, L. B. (2017). Type i interferons and the cytokine TNF cooperatively reprogram the macrophage epigenome to promote inflammatory activation. *Nature Immunology*, *18*(10), 1104–1116. https://doi.org/10.1038/ni.3818

Parks, O. B., Pociask, D. A., Hodzic, Z., Kolls, J. K., & Good, M. (2015). Interleukin-22 Signaling in the Regulation of Intestinal Health and Disease. *Frontiers in Cell and Developmental Biology*, *3*, 85. https://doi.org/10.3389/fcell.2015.00085

Perheentupa, J. (2006). Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy. *The Journal of Clinical Endocrinology & Metabolism*, *91*(8), 2843–2850. https://doi.org/10.1210/jc.2005-2611

Peters, A., Fowler, K. D., Chalmin, F., Merkler, D., Kuchroo, V. K., & Pot, C. (2015). IL-27 Induces Th17 Differentiation in the Absence of STAT1 Signaling. *The Journal of Immunology*, *195*(9), 4144–4153. https://doi.org/10.4049/jimmunol.1302246

Peterson, P., Pitkanen, J., Sillanpaa, N., & Krohn, K. (2004). Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. *Clinical and Experimental Immunology*, *135*(3), 348–357. https://doi.org/10.1111/j.1365-2249.2004.02384.x

Peterson, Pärt, Org, T., & Rebane, A. (2008). Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. *Nature Reviews Immunology*, *8*(12), 948–957. https://doi.org/10.1038/nri2450

Peterson, Pärt, & Peltonen, L. (2005). Autoimmune polyendocrinopathy syndrome type 1 (APS1) and AIRE gene: New views on molecular basis of autoimmunity. *Journal of Autoimmunity*, *25*, 49–55. https://doi.org/10.1016/j.jaut.2005.09.022

Pflanz, S., Hibbert, L., Mattson, J., Rosales, R., Vaisberg, E., Bazan, J. F., Phillips, J. H., McClanahan, T. K., de Waal Malefyt, R., & Kastelein, R. A. (2004). WSX-1 and Glycoprotein 130 Constitute a Signal-Transducing Receptor for IL-27. *The Journal of Immunology*, *172*(4), 2225–2231. https://doi.org/10.4049/jimmunol.172.4.2225

Pflanz, S., Timans, J. C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., De Waal Malefyt, R., … Kastelein, R. A. (2002). IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+T cells. *Immunity*, *16*(6), 779–790. https://doi.org/10.1016/S1074-7613(02)00324-2

Pitt, L. A., Hubert, F.-X., Scott, H. S., Godfrey, D. I., & Berzins, S. P. (2008). NKT cell development in the absence of the autoimmune regulator gene (Aire). *European Journal of Immunology*, *38*(10), 2689–2696. https://doi.org/10.1002/eji.200838553

Plagnol, V., Howson, J. M. M., Smyth, D. J., Walker, N., Hafler, J. P., Wallace, C., Stevens, H., Jackson, L., Simmonds, M. J., Bingley, P. J., Gough, S. C., & Todd, J. A. (2011). Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. *PLoS Genetics*, *7*(8). https://doi.org/10.1371/journal.pgen.1002216

Platanias, L. C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nature Reviews. Immunology*, *5*(5), 375–386. https://doi.org/10.1038/nri1604

Prando, C., Samarina, A., Bustamante, J., Boisson-Dupuis, S., Cobat, A., Picard, C., Alsum, Z., Al-Jumaah, S., Al-Hajjar, S., Frayha, H., Al-Mousa, H., Ben-Mustapha, I., Adimi, P., Feinberg, J., De Suremain, M., Jannière, L., Filipe-Santos, O., Mansouri, N.,

Stephan, J. L., … Casanova, J. L. (2013). Inherited IL-12p40 deficiency: Genetic, immunologic, and clinical features of 49 patients from 30 kindreds. *Medicine (United States)*, *92*(2), 109–122. https://doi.org/10.1097/MD.0b013e31828a01f9

Puel, A., Cypowyj, S., Bustamante, J., Wright, J. F., Liu, L., Lim, H. K., Migaud, M., Israel, L., Chrabieh, M., Audry, M., Gumbleton, M., Toulon, A., Bodemer, C., El-Baghdadi, J., Whitters, M., Paradis, T., Brooks, J., Collins, M., Wolfman, N. M., … Casanova, J.-L. (2011). Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity. *Science*, *332*(6025), 65–68. https://doi.org/10.1126/science.1200439

Puel, Anne, Cypowyj, S., Maródi, L., Abel, L., Picard, C., & Casanova, J.-L. (2012). Inborn errors of human IL-17 immunity underlie chronic mucocutaneous candidiasis. *Current Opinion in Allergy and Clinical Immunology*, *12*(6), 616–622. https://doi.org/10.1097/ACI.0b013e328358cc0b

Puel, Anne, Döffinger, R., Natividad, A., Chrabieh, M., Barcenas-Morales, G., Picard, C., Cobat, A., Ouachée-Chardin, M., Toulon, A., Bustamante, J., Al-Muhsen, S., Al-Owain, M., Arkwright, P. D., Costigan, C., McConnell, V., Cant, A. J., Abinun, M., Polak, M., Bougnères, P.-F., … Casanova, J.-L. (2010). Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *The Journal of Experimental Medicine*, *207*(2), 291–297. https://doi.org/10.1084/jem.20091983

Qiao, Y., Giannopoulou, E. G., Chan, C. H., Park, S. ho, Gong, S., Chen, J., Hu, X., Elemento, O., & Ivashkiv, L. B. (2013). Synergistic activation of inflammatory cytokine genes by interferon-γ-induced chromatin remodeling and toll-like receptor signaling. *Immunity*, *39*(3), 454–469. https://doi.org/10.1016/j.immuni.2013.08.009

Qin, H., Niyongere, S. A., Lee, S. J., Baker, B. J., & Benveniste, E. N. (2008). Expression and Functional Significance of SOCS-1 and SOCS-3 in Astrocytes. *The Journal of Immunology*, *181*(5), 3167–3176. https://doi.org/10.4049/jimmunol.181.5.3167

Radaeva, S., Sun, R., Pan, H. N., Hong, F., & Gao, B. (2004). Interleukin 22 (IL-22) Plays a Protective Role in T Cell-mediated Murine Hepatitis: IL-22 Is a Survival Factor for Hepatocytes via STAT3 Activation. *Hepatology*, *39*(5), 1332–1342. https://doi.org/10.1002/hep.20184

Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., & Vilo, J. (2019). g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Research*, *47*(W1), W191–W198. https://doi.org/10.1093/nar/gkz369

Rautemaa, R., Hietanen, J., Niissalo, S., Pirinen, S., & Perheentupa, J. (2007). Oral and oesophageal squamous cell carcinoma--a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). *Oral Oncology*, *43*(6), 607–613. https://doi.org/10.1016/j.oraloncology.2006.07.005

Rice, G. I., Forte, G. M. A., Szynkiewicz, M., Chase, D. S., Aeby, A., Abdel-Hamid, M. S., Ackroyd, S., Allcock, R., Bailey, K. M., Balottin, U., Barnerias, C., Bernard, G., Bodemer, C., Botella, M. P., Cereda, C., Chandler, K. E., Dabydeen, L., Dale, R. C., De Laet, C., … Crow, Y. J. (2013). Assessment of interferon-related biomarkers in Aicardi-Goutières syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: A case-control study. *The Lancet Neurology*, *12*(12), 1159–1169. https://doi.org/10.1016/S1474-4422(13)70258-8

Rimel, J. K., & Taatjes, D. J. (2018). The essential and multifunctional TFIIH complex. *Protein Science : A Publication of the Protein Society*, *27*(6), 1018–1037. https://doi.org/10.1002/pro.3424

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26. https://doi.org/10.1038/nbt.1754

Rodero, M. P., & Crow, Y. J. (2016). Type I interferon–mediated monogenic autoinflammation: The type I interferonopathies, a conceptual overview. *The Journal of Experimental Medicine*, *213*(12), 2527–2538. https://doi.org/10.1084/jem.20161596

Rodero, M. P., Decalf, J., Bondet, V., Hunt, D., Rice, G. I., Werneke, S., McGlasson, S. L., Alyanakian, M.-A., Bader-Meunier, B., Barnerias, C., Bellon, N., Belot, A., Bodemer, C., Briggs, T. A., Desguerre, I., Frémond, M.-L., Hully, M., van den Maagdenberg, A. M. J. M., Melki, I., … Duffy, D. (2017). Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *The Journal of Experimental Medicine*, *214*(5), 1547–1555. https://doi.org/10.1084/jem.20161451

Rosatelli, M. C., Meloni, A., Meloni, A., Devoto, M., Cao, A., Scott, H. S., Peterson, P., Heino, M., Krohn, K. J., Nagamine, K., Kudoh, J., Shimizu, N., & Antonarakis, S. E. (1998). A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *Human Genetics*, *103*(4), 428–434. http://www.ncbi.nlm.nih.gov/pubmed/9856486

Rösler, B., Wang, X., Keating, S. T., Joosten, L. A. B., Netea, M. G., & van de Veerdonk, F. L. (2018). HDAC inhibitors modulate innate immune responses to microorganisms relevant to chronic mucocutaneous candidiasis. *Clinical and Experimental Immunology*, *194*(2), 205–219. https://doi.org/10.1111/cei.13192

Ross-Innes, C. S., Stark, R., Teschendorff, A. E., Holmes, K. A., Ali, H. R., Dunning, M. J., Brown, G. D., Gojis, O., Ellis, I. O., Green, A. R., Ali, S., Chin, S.-F., Palmieri, C., Caldas, C., & Carroll, J. S. (2012). Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature*, *481*(7381), 389–393. https://doi.org/10.1038/nature10730

Rubino, S. J., Geddes, K., & Girardin, S. E. (2012). Innate IL-17 and IL-22 responses to enteric bacterial pathogens. *Trends in Immunology*, *33*(3), 112–118. https://doi.org/10.1016/J.IT.2012.01.003

Rutz, S., Wang, X., & Ouyang, W. (2014). The IL-20 subfamily of cytokines — from host defence to tissue homeostasis. *Nature Reviews Immunology*, *14*(12), 783–795. https://doi.org/10.1038/nri3766

Ryan, K. R., Hong, M., Arkwright, P. D., Gennery, A. R., Costigan, C., Dominguez, M., Denning, D., McConnell, V., Cant, A. J., Abinun, M., Spickett, G. P., & Lilic, D. (2008). Impaired dendritic cell maturation and cytokine production in patients with chronic mucocutanous candidiasis with or without APECED. *Clinical and Experimental Immunology*, *154*(3), 406–414. https://doi.org/10.1111/j.1365-2249.2008.03778.x

Šahmatova, L., Sügis, E., Šunina, M., Hermann, H., Prans, E., Pihlap, M., Abram, K., Rebane, A., Peterson, H., Peterson, P., Kingo, K., & Kisand, K. (2017). Signs of innate immune activation and premature immunosenescence in psoriasis patients. *Scientific Reports*, *7*(1), 7553. https://doi.org/10.1038/s41598-017-07975-2

Saraiva, M., & O'Garra, A. (2010). The regulation of IL-10 production by immune cells. In *Nature Reviews Immunology* (Vol. 10, Issue 3, pp. 170–181). https://doi.org/10.1038/nri2711

Sarkadi, A. K., Taskó, S., Csorba, G., Tóth, B., Erdős, M., & Maródi, L. (2014). Autoantibodies to IL-17A may be Correlated with the Severity of Mucocutaneous Candidiasis in APECED Patients. *Journal of Clinical Immunology*, *34*(2), 181–193. https://doi.org/10.1007/s10875-014-9987-5

Satoh-Takayama, N., Vosshenrich, C. A. J., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., Mention, J. J., Thiam, K., Cerf-Bensussan, N., Mandelboim, O., Eberl, G., & Di Santo, J. P. (2008). Microbial Flora Drives Interleukin 22 Production in Intestinal NKp46+ Cells that Provide Innate Mucosal Immune Defense. *Immunity*, *29*(6), 958–970. https://doi.org/10.1016/j.immuni.2008.11.001

Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004). Interferon-γ: an overview of signals, mechanisms and functions. *Journal of Leukocyte Biology*, *75*(2), 163–189. https://doi.org/10.1189/jlb.0603252

Severa, M. (2006). Differential responsiveness to IFN- and IFN- of human mature DC through modulation of IFNAR expression. *Journal of Leukocyte Biology*, *79*(6), 1286–1294. https://doi.org/10.1189/jlb.1205742

Shao, S., He, F., Yang, Y., Yuan, G., Zhang, M., & Yu, X. (2012). Th17 cells in type 1 diabetes. In *Cellular Immunology* (Vol. 280, Issue 1, pp. 16–21). https://doi.org/10.1016/j.cellimm.2012.11.001

Sharpe, A. H., Wherry, E. J., Ahmed, R., & Freeman, G. J. (2007). The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. In *Nature Immunology* (Vol. 8, Issue 3, pp. 239–245). https://doi.org/10.1038/ni1443

Shen, F., & Gaffen, S. L. (2008). Structure-function relationships in the IL-17 receptor: Implications for signal transduction and therapy. In *Cytokine* (Vol. 41, Issue 2, pp. 92–104). https://doi.org/10.1016/j.cyto.2007.11.013

Shen, F., Hu, Z., Goswami, J., & Gaffen, S. L. (2006). Identification of common transcriptional regulatory elements in interleukin-17 target genes. *Journal of Biological Chemistry*, *281*(34), 24138–24148. https://doi.org/10.1074/jbc.M604597200

Shih, V. F. S., Cox, J., Kljavin, N. M., Dengler, H. S., Reichelt, M., Kumar, P., Rangell, L., Kolls, J. K., Diehl, L., Ouyang, W., & Ghilardi, N. (2014). Homeostatic IL-23 receptor signaling limits Th17 response through IL-22-mediated containment of commensal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(38), 13942–13947. https://doi.org/10.1073/pnas.1323852111

Shuai, K., & Liu, B. (2003). Regulation of JAK–STAT signalling in the immune system. *Nature Reviews Immunology*, *3*(11), 900–911. https://doi.org/10.1038/nri1226

Smeekens, S. P., Plantinga, T. S., van de Veerdonk, F. L., Heinhuis, B., Hoischen, A., Joosten, L. a B., Arkwright, P. D., Gennery, A., Kullberg, B. J., Veltman, J. a, Lilic, D., van der Meer, J. W. M., & Netea, M. G. (2011). STAT1 hyperphosphorylation and defective IL12R/IL23R signaling underlie defective immunity in autosomal dominant chronic mucocutaneous candidiasis. *PloS One*, *6*(12), e29248. https://doi.org/10.1371/journal.pone.0029248

Smyth, A. E., Kaleviste, E., Snow, A., Kisand, K., McMahon, C. J., Cant, A. J., & Leahy, T. R. (2018). Aortic Calcification in a Patient with a Gain-of-Function STAT1 Mutation. *Journal of Clinical Immunology*, *38*(4), 468–470. https://doi.org/10.1007/s10875-018-0513-z

Sobh, A., Chou, J., Schneider, L., Geha, R. S., & Massaad, M. J. (2016). Chronic mucocutaneous candidiasis associated with an SH2 domain gain-of-function mutation that enhances STAT1 phosphorylation. *Journal of Allergy and Clinical Immunology*, *138*(1), 297–299. https://doi.org/10.1016/j.jaci.2015.12.1320

Sobkowiak, M. J., Davanian, H., Heymann, R., Gibbs, A., Emgård, J., Dias, J., Aleman, S., Krüger-Weiner, C., Moll, M., Tjernlund, A., Leeansyah, E., Sällberg Chen, M., & Sandberg, J. K. (2019). Tissue-resident MAIT cell populations in human oral mucosa exhibit an activated profile and produce IL-17. *European Journal of Immunology*, *49*(1), 133–143. https://doi.org/10.1002/eji.201847759

Song, M. M., & Shuai, K. (1998). The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and anti-proliferative activities. *Journal of Biological Chemistry*, *273*(52), 35056–35062. https://doi.org/10.1074/jbc.273.52.35056

Sonnenberg, G. F., Monticelli, L. A., Alenghat, T., Fung, T. C., Hutnick, N. A., Kunisawa, J., Shibata, N., Grunberg, S., Sinha, R., Zahm, A. M., Tardif, M. R., Sathaliyawala, T., Kubota, M., Farber, D. L., Collman, R. G., Shaked, A., Fouser, L. A., Weiner, D. B., Tessier, P. A., … Artis, D. (2012). Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science*, *336*(6086), 1321–1325. https://doi.org/10.1126/science.1222551

Spolski, R., & Leonard, W. J. (2008). Interleukin-21: Basic Biology and Implications for Cancer and Autoimmunity. *Annual Review of Immunology*, *26*(1), 57–79. https://doi.org/10.1146/annurev.immunol.26.021607.090316

Stark, G. R., & Darnell, J. E. (2012). The JAK-STAT Pathway at Twenty. In *Immunity* (Vol. 36, Issue 4, pp. 503–514). https://doi.org/10.1016/j.immuni.2012.03.013

Stellacci, E., Moneta, G. M., Bruselles, A., Barresi, S., Pizzi, S., Torre, G., De Benedetti, F., Tartaglia, M., & Insalaco, A. (2019). The activating p.Ser466Arg change in STAT1 causes a peculiar phenotype with features of interferonopathies. *Clinical Genetics*, *96*(6), 585–589. https://doi.org/10.1111/cge.13632

Stetson, D. B., & Medzhitov, R. (2006). Type I Interferons in Host Defense. In *Immunity* (Vol. 25, Issue 3, pp. 373–381). https://doi.org/10.1016/j.immuni.2006.08.007

Stolarski, B., Pronicka, E., Korniszewski, L., Pollak, A., Kostrzewa, G., Rowińska, E., Włodarski, P., Skórka, A., Gremida, M., Krajewski, P., & Ploski, R. (2006). Molecular background of polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome in a Polish population: novel AIRE mutations and an estimate of disease prevalence. *Clinical Genetics*, *70*(4), 348–354. https://doi.org/10.1111/j.1399-0004.2006.00690.x

Stumhofer, J. S., Laurence, A., Wilson, E. H., Huang, E., Tato, C. M., Johnson, L. M., Villarino, A. V., Huang, Q., Yoshimura, A., Sehy, D., Saris, C. J. M., O'Shea, J. J., Hennighausen, L., Ernst, M., & Hunter, C. A. (2006). Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nature Immunology*, *7*(9), 937–945. https://doi.org/10.1038/ni1376

Sugimoto, K., Ogawa, A., Mizoguchi, E., Shimomura, Y., Andoh, A., Bhan, A. K., Blumberg, R. S., Xavier, R. J., & Mizoguchi, A. (2008). IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *Journal of Clinical Investigation*, *118*(2), 534–544. https://doi.org/10.1172/JCI33194

Swaidani, S., Liu, C., Zhao, J., Bulek, K., & Li, X. (2019). TRAF regulation of IL-17 cytokine signaling. *Frontiers in Immunology*, *10*(JUN). https://doi.org/10.3389/fimmu.2019.01293

Swiecki, M., & Colonna, M. (2015). The multifaceted biology of plasmacytoid dendritic cells. In *Nature Reviews Immunology* (Vol. 15, Issue 8, pp. 471–485). Nature Publishing Group. https://doi.org/10.1038/nri3865

Takeda, A., Hamano, S., Yamanaka, A., Hanada, T., Ishibashi, T., Mak, T. W., Yoshimura, A., & Yoshida, H. (2003). Cutting Edge: Role of IL-27/WSX-1 Signaling for Induction of T-Bet Through Activation of STAT1 During Initial Th1 Commitment. *The Journal of Immunology*, *170*(10), 4886–4890. https://doi.org/10.4049/jimmunol.170.10.4886

Takezaki, S., Yamada, M., Kato, M., Park, M.-J., Maruyama, K., Yamazaki, Y., Chida, N., Ohara, O., Kobayashi, I., & Ariga, T. (2012). Chronic mucocutaneous candidiasis

caused by a gain-of-function mutation in the STAT1 DNA-binding domain. *Journal of Immunology (Baltimore, Md. : 1950)*, *189*(3), 1521–1526. https://doi.org/10.4049/jimmunol.1200926

Tan, F. K., Zhou, X., Mayes, M. D., Gourh, P., Guo, X., Marcum, C., Jin, L., & Arnett Jr, F. C. (2006). Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. *Rheumatology*, *45*, 694–702. https://doi.org/10.1093/rheumatology/kei244

Tang, C., Kakuta, S., Shimizu, K., Kadoki, M., Kamiya, T., Shimazu, T., Kubo, S., Saijo, S., Ishigame, H., Nakae, S., & Iwakura, Y. (2018). Suppression of IL-17F, but not of IL-17A, provides protection against colitis by inducing T reg cells through modification of the intestinal microbiota. *Nature Immunology*, *19*(7), 755–765. https://doi.org/10.1038/s41590-018-0134-y

Thyrell, L., Arulampalam, V., Hjortsberg, L., Farnebo, M., Grandér, D., & Pokrovskaja Tamm, K. (2007). Interferon alpha induces cell death through interference with interleukin 6 signaling and inhibition of STAT3 activity. *Experimental Cell Research*, *313*(19), 4015–4024. https://doi.org/10.1016/J.YEXCR.2007.08.007

Toubiana, J., Okada, S., Hiller, J., Oleastro, M., Gomez, M. L., Becerra, J. C. A., Ouachée-Chardin, M., Fouyssac, F., Girisha, K. M., Etzioni, A., Van Montfrans, J., Camcioglu, Y., Kerns, L. A., Belohradsky, B., Blanche, S., Bousfiha, A., Rodriguez-Gallego, C., Meyts, I., Kisand, K., … Puel, A. (2016). Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood*, *127*(25), 3154–3164. https://doi.org/10.1182/blood-2015-11-679902

Trebušak Podkrajšek, K., Bratanič, N., Kržišnik, C., & Battelino, T. (2005). Autoimmune Regulator-1 Messenger Ribonucleic Acid Analysis in a Novel Intronic Mutation and Two Additional Novel *AIRE* Gene Mutations in a Cohort of Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Patients. *The Journal of Clinical Endocrinology & Metabolism*, *90*(8), 4930–4935. https://doi.org/10.1210/jc.2005-0418

Trinchieri, G. (2010). Type I interferon: Friend or foe? In *Journal of Experimental Medicine* (Vol. 207, Issue 10, pp. 2053–2063). https://doi.org/10.1084/jem.20101664

Tripathi, S. K., Chen, Z., Larjo, A., Kanduri, K., Nousiainen, K., Äijo, T., Ricaño-Ponce, I., Hrdlickova, B., Tuomela, S., Laajala, E., Salo, V., Kumar, V., Wijmenga, C., Lähdesmäki, H., & Lahesmaa, R. (2017). Genome-wide Analysis of STAT3-Mediated Transcription during Early Human Th17 Cell Differentiation. *Cell Reports*, *19*(9), 1888–1901. https://doi.org/10.1016/j.celrep.2017.05.013

Tuovinen, H., Pöntynen, N., Gylling, M., Kekäläinen, E., Perheentupa, J., Miettinen, A., & Arstila, T. P. (2009). γδ T cells develop independently of Aire. *Cellular Immunology*, *257*(1–2), 5–12. https://doi.org/10.1016/j.cellimm.2009.01.012

Turner, J.-E., Stockinger, B., & Helmby, H. (2013). IL-22 Mediates Goblet Cell Hyperplasia and Worm Expulsion in Intestinal Helminth Infection. *PLoS Pathogens*, *9*(10), e1003698. https://doi.org/10.1371/journal.ppat.1003698

Uonaga, T., Yoshida, K., Harada, T., Shimodahira, M., & Nakamura, Y. (2012). Case of type 1 diabetes mellitus following interferon β-1a treatment for multiple sclerosis. *Internal Medicine*, *51*(14), 1875–1877. https://doi.org/10.2169/internalmedicine.51.7609

Uzel, G., Sampaio, E. P., Lawrence, M. G., Hsu, A. P., Hackett, M., Dorsey, M. J., Noel, R. J., Verbsky, J. W., Freeman, A. F., Janssen, E., Bonilla, F. A., Pechacek, J., Chandrasekaran, P., Browne, S. K., Agharahimi, A., Gharib, A. M., Mannurita, S. C., Yim, J. J., Gambineri, E., … Holland, S. M. (2013). Dominant gain-of-function

STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *The Journal of Allergy and Clinical Immunology*, *131*(6), 1611–1623. https://doi.org/10.1016/j.jaci.2012.11.054

Vahedi, G., Takahashi, H., Nakayamada, S., Sun, H., Sartorelli, V., Kanno, Y., & O'Shea, J. J. (2012). STATs Shape the Active Enhancer Landscape of T Cell Populations. *Cell*, *151*(5), 981–993. https://doi.org/10.1016/j.cell.2012.09.044

van de Veerdonk, F. L., Plantinga, T. S., Hoischen, A., Smeekens, S. P., Joosten, L. a B., Gilissen, C., Arts, P., Rosentul, D. C., Carmichael, A. J., Smits-van der Graaf, C. a a, Kullberg, B. J., van der Meer, J. W. M., Lilic, D., Veltman, J. a, & Netea, M. G. (2011). STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *The New England Journal of Medicine*, *365*(1), 54–61. https://doi.org/10.1056/NEJMoa1100102

Vargas-Hernández, A., Mace, E. M., Zimmerman, O., Zerbe, C. S., Freeman, A. F., Rosenzweig, S., Leiding, J. W., Torgerson, T., Altman, M. C., Schussler, E., Cunningham-Rundles, C., Chinn, I. K., Carisey, A. F., Hanson, I. C., Rider, N. L., Holland, S. M., Orange, J. S., & Forbes, L. R. (2018). Ruxolitinib partially reverses functional natural killer cell deficiency in patients with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations. *Journal of Allergy and Clinical Immunology*, *141*(6), 2142-2155.e5. https://doi.org/10.1016/j.jaci.2017.08.040

Veldhoen, M., Hirota, K., Westendorf, A. M., Buer, J., Dumoutier, L., Renauld, J.-C., & Stockinger, B. (2008). The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature*, *453*(7191), 106–109. https://doi.org/10.1038/nature06881

Villarino, A. V., Huang, E., & Hunter, C. A. (2004). Understanding the Pro- and Anti-Inflammatory Properties of IL-27. *The Journal of Immunology*, *173*(2), 715–720. https://doi.org/10.4049/jimmunol.173.2.715

Villarino, A. V, Kanno, Y., & O'Shea, J. J. (2017). Mechanisms and consequences of Jak–STAT signaling in the immune system. *Nature Immunology*, *18*(4), 374–384. https://doi.org/10.1038/ni.3691

Wan, C.-K., Andraski, A. B., Spolski, R., Li, P., Kazemian, M., Oh, J., Samsel, L., Swanson, P. A., McGavern, D. B., Sampaio, E. P., Freeman, A. F., Milner, J. D., Holland, S. M., & Leonard, W. J. (2015). Opposing roles of STAT1 and STAT3 in IL-21 function in CD4+ T cells. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), 9394–9399. https://doi.org/10.1073/pnas.1511711112

Wang, R., Han, G., Wang, J., Chen, G., Xu, R., Wang, L., Li, X., Shen, B., & Li, Y. (2008). The pathogenic role of interleukin-27 in autoimmune diabetes. *Cellular and Molecular Life Sciences*, *65*(23), 3851–3860. https://doi.org/10.1007/s00018-008-8540-1

Wei, L., Laurence, A., Elias, K. M., & O'Shea, J. J. (2007). IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *Journal of Biological Chemistry*, *282*(48), 34605–34610. https://doi.org/10.1074/jbc.M705100200

Wei, L., Vahedi, G., Sun, H.-W., Watford, W. T., Takatori, H., Ramos, H. L., Takahashi, H., Liang, J., Gutierrez-Cruz, G., Zang, C., Peng, W., O'Shea, J. J., & Kanno, Y. (2010). Discrete Roles of STAT4 and STAT6 Transcription Factors in Tuning Epigenetic Modifications and Transcription during T Helper Cell Differentiation. *Immunity*, *32*(6), 840–851. https://doi.org/10.1016/j.immuni.2010.06.003

Weinacht, K. G., Charbonnier, L.-M., Alroqi, F., Plant, A., Qiao, Q., Wu, H., Ma, C., Torgerson, T. R., Rosenzweig, S. D., Fleisher, T. A., Notarangelo, L. D., Hanson, I. C., Forbes, L. R., & Chatila, T. A. (2017). Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *The Journal of Allergy and Clinical Immunology*, *139*(5), 1629-1640.e2. https://doi.org/10.1016/j.jaci.2016.11.022

Willcox, A., Richardson, S. J., Bone, A. J., Foulis, A. K., & Morgan, N. G. (2009). Analysis of islet inflammation in human type 1 diabetes. *Clinical and Experimental Immunology*, *155*(2), 173–181. https://doi.org/10.1111/j.1365-2249.2008.03860.x

Witte, E., Witte, K., Warszawska, K., Sabat, R., & Wolk, K. (2010). Interleukin-22: A cytokine produced by T, NK and NKT cell subsets, with importance in the innate immune defense and tissue protection. *Cytokine & Growth Factor Reviews*, *21*(5), 365–379. https://doi.org/10.1016/j.cytogfr.2010.08.002

Wolff, A. S. B., Sarkadi, A. K., Maródi, L., Kärner, J., Orlova, E., Oftedal, B. E. V., Kisand, K., Oláh, É., Meloni, A., Myhre, A. G., Husebye, E. S., Motaghedi, R., Perheentupa, J., Peterson, P., Willcox, N., & Meager, A. (2013). Anti-Cytokine Autoantibodies Preceding Onset of Autoimmune Polyendocrine Syndrome Type I Features in Early Childhood. *Journal of Clinical Immunology*, *33*(8), 1341–1348. https://doi.org/10.1007/s10875-013-9938-6

Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K., & Sabat, R. (2004). IL-22 increases the innate immunity of tissues. *Immunity*, *21*(2), 241–254. https://doi.org/10.1016/j.immuni.2004.07.007

Wolk, K., Witte, E., Hoffmann, U., Doecke, W.-D., Endesfelder, S., Asadullah, K., Sterry, W., Volk, H.-D., Wittig, B. M., & Sabat, R. (2007). IL-22 Induces Lipopolysaccharide-Binding Protein in Hepatocytes: A Potential Systemic Role of IL-22 in Crohn's Disease. *The Journal of Immunology*, *178*(9), 5973–5981. https://doi.org/10.4049/jimmunol.178.9.5973

Wolk, K., Witte, E., Wallace, E., Döcke, W. D., Kunz, S., Asadullah, K., Volk, H. D., Sterry, W., & Sabat, R. (2006). IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: A potential role in psoriasis. *European Journal of Immunology*, *36*(5), 1309–1323. https://doi.org/10.1002/eji.200535503

Xu, W., Presnell, S. R., Parrish-Novak, J., Kindsvogel, W., Jaspers, S., Chen, Z., Dillon, S. R., Gao, Z., Gilbert, T., Madden, K., Schlutsmeyer, S., Yao, L., Whitmore, T. E., Chandrasekher, Y., Grant, F. J., Maurer, M., Jelinek, L., Storey, H., Brender, T., … Foster, D. C. (2001). A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(17), 9511–9516. https://doi.org/10.1073/pnas.171303198

Yamazaki, Y., Yamada, M., Kawai, T., Morio, T., Onodera, M., Ueki, M., Watanabe, N., Takada, H., Takezaki, S., Chida, N., Kobayashi, I., & Ariga, T. (2014). Two Novel Gain-of-Function Mutations of STAT1 Responsible for Chronic Mucocutaneous Candidiasis Disease: Impaired Production of IL-17A and IL-22, and the Presence of Anti–IL-17F Autoantibody. *The Journal of Immunology*, *193*(10), 4880–4887. https://doi.org/10.4049/jimmunol.1401467

Yang, S., Fujikado, N., Kolodin, D., Benoist, C., & Mathis, D. (2015). Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. *Science*, *348*(6234), 589–594. https://doi.org/10.1126/science.aaa7017

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., Ma, L., Shah, B., Panopoulos, A. D., Schluns, K. S., Watowich, S. S., Tian, Q., Jetten, A. M., & Dong, C. (2008). T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors RORα and RORγ. *Immunity*, *28*(1), 29–39. https://doi.org/10.1016/j.immuni.2007.11.016

Ye, P., Garvey, P. B., Zhang, P., Nelson, S., Bagby, G., Summer, W. R., Schwarzenberger, P., Shellito, J. E., & Kolls, J. K. (2001). Interleukin-17 and lung host defense against klebsiella pneumoniae infection. *American Journal of Respiratory Cell and Molecular Biology*, *25*(3), 335–340. https://doi.org/10.1165/ajrcmb.25.3.4424

Ye, P., Rodriguez, F. H., Kanaly, S., Stocking, K. L., Schurr, J., Schwarzenberger, P., Oliver, P., Huang, W., Zhang, P., Zhang, J., Shellito, J. E., Bagby, G. J., Nelson, S., Charrier, K., Peschon, J. J., & Kolls, J. K. (2001). Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *Journal of Experimental Medicine*, *194*(4), 519–527. https://doi.org/10.1084/jem.194.4.519

Yoshimura, A., Suzuki, M., Sakaguchi, R., Hanada, T., & Yasukawa, H. (2012). SOCS, inflammation, and autoimmunity. In *Frontiers in Immunology* (Vol. 3, Issue MAR, p. 20). https://doi.org/10.3389/fimmu.2012.00020

Yu, G., Wang, L.-G., & He, Q.-Y. (2015). ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics*, *31*(14), 2382–2383. https://doi.org/10.1093/bioinformatics/btv145

Zenewicz, L. A., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A. J., Stevens, S., & Flavell, R. A. (2008). Innate and Adaptive Interleukin-22 Protects Mice from Inflammatory Bowel Disease. *Immunity*, *29*(6), 947–957. https://doi.org/10.1016/j.immuni.2008.11.003

Zenewicz, L. A., Yin, X., Wang, G., Elinav, E., Hao, L., Zhao, L., & Flavell, R. A. (2013). IL-22 Deficiency Alters Colonic Microbiota To Be Transmissible and Colitogenic. *The Journal of Immunology*, *190*(10), 5306–5312. https://doi.org/10.4049/jimmunol.1300016

Zenobia, C., & Hajishengallis, G. (2015). Basic biology and role of interleukin-17 in immunity and inflammation. *Periodontology 2000*, *69*(1), 142–159. https://doi.org/10.1111/prd.12083

Zhang, J., Hung, G.-C., Nagamine, K., Li, B., Tsai, S., & Lo, S.-C. (2016). Development of Candida-Specific Real-Time PCR Assays for the Detection and Identification of Eight Medically Important Candida Species. *Microbiology Insights*, *9*, 21–28. https://doi.org/10.4137/MBI.S38517

Zhang, L. J., & Gallo, R. L. (2016). Antimicrobial peptides. In *Current Biology* (Vol. 26, Issue 1, pp. R14–R19). Cell Press. https://doi.org/10.1016/j.cub.2015.11.017

Zhang, Y., Ma, C. A., Lawrence, M. G., Break, T. J., O'Connell, M. P., Lyons, J. J., López, D. B., Barber, J. S., Zhao, Y., Barber, D. L., Freeman, A. F., Holland, S. M., Lionakis, M. S., & Milner, J. D. (2017). PD-L1 up-regulation restrains Th17 cell differentiation in *STAT3* loss- and *STAT1* gain-of-function patients. *The Journal of Experimental Medicine*, *214*(9), 2523–2533. https://doi.org/10.1084/jem.20161427

Zheng, J., van de Veerdonk, F. L., Crossland, K. L., Smeekens, S. P., Chan, C. M., Al Shehri, T., Abinun, M., Gennery, A. R., Mann, J., Lendrem, D. W., Netea, M. G., Rowan, A. D., & Lilic, D. (2015). Gain-of-function STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). *European Journal of Immunology*, *45*(10), 2834–2846. https://doi.org/10.1002/eji.201445344

Zheng, S. G., Wang, J., & Horwitz, D. A. (2008). Cutting Edge: Foxp3 + CD4 + CD25 + Regulatory T Cells Induced by IL-2 and TGF-β Are Resistant to Th17 Conversion by IL-6. *The Journal of Immunology*, *180*(11), 7112–7116. https://doi.org/10.4049/jimmunol.180.11.7112

Zheng, Y., Danilenko, D. M., Valdez, P., Kasman, I., Eastham-Anderson, J., Wu, J., & Ouyang, W. (2007). Interleukin-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*, *445*(7128), 648–651. https://doi.org/10.1038/nature05505

Zhong, M., Henriksen, M. A., Takeuchi, K., Schaefer, O., Liu, B., ten Hoeve, J., Ren, Z., Mao, X., Chen, X., Shuai, K., Darnell, J. E., & Jr. (2005). Implications of an anti-parallel dimeric structure of nonphosphorylated STAT1 for the activation-inactivation cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(11), 3966–3971. https://doi.org/10.1073/pnas.0501063102

Zimmerman, O., Olbrich, P., Freeman, A. F., Rosen, L. B., Uzel, G., Zerbe, C. S., Rosenzweig, S. D., Kuehn, H. S., Holmes, K. L., Stephany, D., Ding, L., Sampaio, E. P., Hsu, A. P., & Holland, S. M. (2019). STAT1 gain-of-function mutations cause high total STAT1 levels with normal dephosphorylation. *Frontiers in Immunology*, *10*(JULY). https://doi.org/10.3389/fimmu.2019.01433

Zimmerman, O., Rösler, B., Zerbe, C. S., Rosen, L. B., Hsu, A. P., Uzel, G., Freeman, A. F., Sampaio, E. P., Rosenzweig, S. D., Kuehn, H. S., Kim, T., Brooks, K. M., Kumar, P., Wang, X., Netea, M. G., van de Veerdonk, F. L., & Holland, S. M. (2017). Risks of Ruxolitinib in STAT1 Gain-of-Function-Associated Severe Fungal Disease. *Open Forum Infectious Diseases*, *4*(4), ofx202. https://doi.org/10.1093/ofid/ofx202

Zindl, C. L., Lai, J.-F., Lee, Y. K., Maynard, C. L., Harbour, S. N., Ouyang, W., Chaplin, D. D., & Weaver, C. T. (2013). IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proceedings of the National Academy of Sciences*, *110*(31), 12768–12773. https://doi.org/10.1073/pnas.1300318110

Zlotogora, J., & Shapiro, M. S. (1992). Polyglandular autoimmune syndrome type I among Iranian Jews. *Journal of Medical Genetics*, *29*(11), 824–826. https://doi.org/10.1136/jmg.29.11.824

11. ACKNOWLEDGMENTS

The research presented in this thesis was performed at the Molecular Pathology Research Group, Institute of Biomedicine and Translational Medicine at the University of Tartu. I would like to express my most sincere gratitude to the people who supported me throughout my doctorate studies:

- my supervisors Kai Kisand, Pärt Peterson, and Lili Milani, who have guided and encouraged me along my academic path. Especially Kai Kisand, who has been my intellectual mentor for years with her everlasting knowledge and cheerful presence;

- all my wonderful colleagues from our laboratory for being always friendly, helpful and hardworking;

- my referees Katrin Õunap and Reet Mändar, for their valuable comments and suggestions on the manuscript;

- my opponent Tadej Avcin, for agreeing to provide scientific and intellectual criticism;

- all the co-authors of my scientific publications and collaborators for their valuable contributions;

- and finally, I would like to especially thank my dear family and precious friends for being always motivative, wonderful, and cheering. For staying by my side throughout this journey!

PUBLICATIONS

CURRICULUM VITAE

Name: Epp Kaleviste
Born: April 15th, 1990, Tallinn, Estonia
Citizenship: Estonian
Address: Molecular Pathology Research Group,
Institute of Biomedicine and Translational Medicine,
University of Tartu, Ravila 19, 50411, Tartu
Telephone: +372 737 4186
Email: eppkaleviste@gmail.com

Education:

2006–2009	Collegium Educationis Revaliae
2009–2012	University of Tartu, Faculty of Science and Technology, Genetic engineering, BSc
2012–2015	University of Tartu, Biomedicine, MSc
2015–2020	University of Tartu, Medicine, PhD

Institution and position:

2014–2019	University of Tartu, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, Molecular pathology study group, laboratory specialist
2018−...	University of Tartu, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, Molecular pathology study group, junior research fellow

R&D related managerial and administrative work:

2018−...	Estonian Society for Immunology and Allergology

Publications:

1. Kasela, S., Kisand, K., Tserel, L., **Kaleviste, E.**, Remm, A., Fischer, K., Esko, T., Westra, H. J., Fairfax, B. P., Makino, S., Knight, J. C., Franke, L., Metspalu, A., Peterson, P., and Milani, L. (2017). Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. *PLoS Genetics*, 13(3), e1006643.
2. Smyth, A. E., **Kaleviste, E.**, Snow, A., Kisand, K., McMahon, C. J., Cant, A. J., and Leahy, T. R. (2018). Aortic calcification in a patient with a gain-of-function STAT1 mutation. *Journal of Clinical Immunology*, 38(4), 468–470.
3. Šunina, M., **Kaleviste, E.**, Uibo, R., and Kisand, K. (2018). Unstimulated Adult Human B Cells Include an IL-10+ Population with Suppressive Properties and an Activated Phenotype. *Cytometry Part A*, 93(11), 1150–1156.
4. Raam, L., **Kaleviste, E.**, Šunina, M., Vaher, H., Saare, M., Prans, E., Pihlap, M., Abram, K., Karelson, M., Peterson, P., Rebane, A., Kisand, K., and Kingo, K. (2018). Lymphoid stress surveillance response contributes to vitiligo pathogenesis. *Frontiers in Immunology*, 9, 2707.

5. **Kaleviste, E.**, Saare, M., Leahy, T. R., Bondet, V., Duffy, D., Mogensen, T. H., Jørgensen, S. E., Nurm, H., Ip, W., Davies, E. G., Sauer, S., Syvänen, A. C., Milani, L., Peterson, P., and Kisand, K. (2019). Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically determined. *European Journal of Immunology*, 49(5), 790–800.
6. **Kaleviste, E.**, Rühlemann, M., Kärner, J., Haljasmägi, L., Tserel, L., Org, E., Trebušak Podkrajšek, K., Battelino, T., Bang, C., Franke, A., Peterson, P., and Kisand, K. (2020). IL-22 paucity in APECED is associated with mucosal and microbial alterations in oral cavity. *Frontiers in Immunology*, 11, 838.

ELULOOKIRJELDUS

Nimi: Epp Kaleviste
Sündinud: 15. aprill 1990, Tallinn, Eesti
Kodakondsus: eestlane
Aadress: Molekulaarpatoloogia uurimisgrupp,
Bio-ja siirdemeditsiini instituut, Tartu Ülikool,
Ravila 19, 50411, Tartu, Eesti
Telefon: +372 737 4186
E-mail: eppkaleviste@gmail.com

Hariduskäik:

2006–2009	Vanalinna Hariduskolleegium
2009–2012	Tartu Ülikool, geenitehnoloogia, BSc
2012–2015	Tartu Ülikool, biomeditsiin, M.Sc
2015–2020	Tartu Ülikool, arstiteaduse doktoriõpe

Teenistuskäik:

2014–2019	Tartu Ülikool; Bio-ja siirdemeditsiini instituut, Molekulaarpatoloogia töögrupp, laborispetsialist (0.50)
2018−...	Tartu Ülikool; Bio-ja siirdemeditsiini instituut, Molekulaarpatoloogia töögrupp, nooremteadur (0.50)

Teadusorganisatsiooniline ja -administratiivne tegevus:

2018–...	Eesti Immunoloogide ja Allergoloogide Selts

Publikatsioonid:

1. Kasela, S., Kisand, K., Tserel, L., **Kaleviste, E.**, Remm, A., Fischer, K., Esko, T., Westra, H. J., Fairfax, B. P., Makino, S., Knight, J. C., Franke, L., Metspalu, A., Peterson, P., and Milani, L. (2017). Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. *PLoS Genetics*, 13(3), e1006643.
2. Smyth, A. E., **Kaleviste, E.**, Snow, A., Kisand, K., McMahon, C. J., Cant, A. J., and Leahy, T. R. (2018). Aortic calcification in a patient with a gain-of-function STAT1 mutation. *Journal of Clinical Immunology*, 38(4), 468–470.
3. Šunina, M., **Kaleviste, E.**, Uibo, R., and Kisand, K. (2018). Unstimulated Adult Human B Cells Include an IL-10+ Population with Suppressive Properties and an Activated Phenotype. *Cytometry Part A*, 93(11), 1150–1156.
4. Raam, L., **Kaleviste, E.**, Šunina, M., Vaher, H., Saare, M., Prans, E., Pihlap, M., Abram, K., Karelson, M., Peterson, P., Rebane, A., Kisand, K., and Kingo, K. (2018). Lymphoid stress surveillance response contributes to vitiligo pathogenesis. *Frontiers in Immunology*, 9, 2707.

5. **Kaleviste, E.**, Saare, M., Leahy, T. R., Bondet, V., Duffy, D., Mogensen, T. H., Jørgensen, S. E., Nurm, H., Ip, W., Davies, E. G., Sauer, S., Syvänen, A. C., Milani, L., Peterson, P., and Kisand, K. (2019). Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically determined. *European Journal of Immunology*, 49(5), 790–800.
6. **Kaleviste, E.**, Rühlemann, M., Kärner, J., Haljasmägi, L., Tserel, L., Org, E., Trebušak Podkrajšek, K., Battelino, T., Bang, C., Franke, A., Peterson, P., and Kisand, K. (2020). IL-22 paucity in APECED is associated with mucosal and microbial alterations in oral cavity. *Frontiers in Immunology*, 11, 838.

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos**. The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar**. Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik**. Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu**. Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar**. Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus**. Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar**. Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm**. On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross**. Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo**. Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik**. The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa**. Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk**. Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar**. Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi**. The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar**. Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang**. The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak**. Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki**. Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar**. Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel**. Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik**. Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk**. Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets**. Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik**. Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi**. Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo**. Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber**. *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla**. Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk**. Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf**. Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv**. Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi**. Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva**. Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin**. Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev**. Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik**. Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto**. Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar**. Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge**. Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg**. The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp**. Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots**. Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe**. Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv**. Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan**. Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik**. Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand**. Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi**. Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask**. Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson**. Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar**. Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl**. Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap**. Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg**. *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro**. Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi**. Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann**. Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke**. Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves**. Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda**. Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist**. Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik**. Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull**. Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos**. Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova**. Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda**. Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner**. *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi**. Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla**. Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas**. Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk**. Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen**. Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar**. Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep**. Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes**. Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu**. Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi**. Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa**. Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk**. Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.
84. **Helena Soomer**. Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin**. Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann**. Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu**. Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas**. Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba**. Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev**. CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja**. Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla**. Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid**. Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter**. Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa**. Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre**. Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev**. Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema**. Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet**. Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.
102. **Eduard Maron**. Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona**. *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk**. Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv**. Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun**. Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda**. Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo**. The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila**. Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla**. Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell**. Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme**. Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor**. Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm**. Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite**. Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll**. Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach**. Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell**. Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako**. Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtson**. Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld**. Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann**. Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar**. Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson**. Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus**. Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder**. Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru**. Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand**. Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo**. Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo**. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild**. Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik**. Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller**. Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik**. Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals**. Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus**. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab**. Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme**. Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin**. The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik**. Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu**. Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu**. Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik**. Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets**. The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits**. Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam**. Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots**. Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp**. The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull**. Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre**. Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link**. Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre**. Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak**. Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich**. Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep**. Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk**. Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass**. L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai**. Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum**. Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova**. The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi**. Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi**. A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe**. Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm**. Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu**. Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont**. Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt**. Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru**. Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov**. Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg**. Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm**. Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg**. Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal**. Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass**. Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu**. Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.
205. **Oksana Jagur**. Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk**. Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal**. Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand**. Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma**. Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre**. Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp**. Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum**. Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson**. Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand**. Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma**. Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg**. Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit**. Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees**. Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik**. The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel**. Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna**. The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrid Liiv**. Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison**. Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress**. Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru**. Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu**. Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets**. The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome**. Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo**. Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin**. Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna**. Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase**. Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel**. Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere**. Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust**. Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman**. Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar**. Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson**. Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme**. High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part**. Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.
241. **Urve Paaver**. New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.
242. **Aleksandr Peet**. Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.
243. **Piret Mitt**. Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.
244. **Merli Saare**. Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.
245. **Kaja-Triin Laisaar**. People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.
246. **Eero Merilind**. Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.
247. **Jaanika Kärner**. Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.
248. **Kaido Paapstel**. Metabolomic profile of arterial stiffness and early biomarkers of renal damage in atherosclerosis. Tartu, 2016, 173 p.
249. **Liidia Kiisk**. Long-term nutritional study: anthropometrical and clinico-laboratory assessments in renal replacement therapy patients after intensive nutritional counselling. Tartu, 2016, 207 p.
250. **Georgi Nellis**. The use of excipients in medicines administered to neonates in Europe. Tartu, 2017, 159 p.
251. **Aleksei Rakitin**. Metabolic effects of acute and chronic treatment with valproic acid in people with epilepsy. Tartu, 2017, 125 p.
252. **Eveli Kallas**. The influence of immunological markers to susceptibility to HIV, HBV, and HCV infections among persons who inject drugs. Tartu, 2017, 138 p.
253. **Tiina Freimann**. Musculoskeletal pain among nurses: prevalence, risk factors, and intervention. Tartu, 2017, 125 p.
254. **Evelyn Aaviksoo**. Sickness absence in Estonia: determinants and influence of the sick-pay cut reform. Tartu, 2017, 121 p.
255. **Kalev Nõupuu**. Autosomal-recessive Stargardt disease: phenotypic heterogeneity and genotype-phenotype associations. Tartu, 2017, 131 p.
256. **Ho Duy Binh**. Osteogenesis imperfecta in Vietnam. Tartu, 2017, 125 p.
257. **Uku Haljasorg**. Transcriptional mechanisms in thymic central tolerance. Tartu, 2017, 147 p.
258. **Živile Riispere**. IgA Nephropathy study according to the Oxford Classification: IgA Nephropathy clinical-morphological correlations, disease progression and the effect of renoprotective therapy. Tartu, 2017, 129 p.
259. **Hiie Soeorg**. Coagulase-negative staphylococci in gut of preterm neonates and in breast milk of their mothers. Tartu, 2017, 216 p.

260. **Anne-Mari Anton Willmore**. Silver nanoparticles for cancer research. Tartu, 2017, 132 p.
261. **Ott Laius**. Utilization of osteoporosis medicines, medication adherence and the trend in osteoporosis related hip fractures in Estonia. Tartu, 2017, 134 p.
262. **Alar Aab**. Insights into molecular mechanisms of asthma and atopic dermatitis. Tartu, 2017, 164 p.
263. **Sander Pajusalu**. Genome-wide diagnostics of Mendelian disorders: from chromosomal microarrays to next-generation sequencing. Tartu, 2017, 146 p.
264. **Mikk Jürisson**. Health and economic impact of hip fracture in Estonia. Tartu, 2017, 164 p.
265. **Kaspar Tootsi**. Cardiovascular and metabolomic profiling of osteoarthritis. Tartu, 2017, 150 p.
266. **Mario Saare**. The influence of AIRE on gene expression – studies of transcriptional regulatory mechanisms in cell culture systems. Tartu, 2017, 172 p.
267. **Piia Jõgi**. Epidemiological and clinical characteristics of pertussis in Estonia. Tartu, 2018, 168 p.
268. **Elle Põldoja**. Structure and blood supply of the superior part of the shoulder joint capsule. Tartu, 2018, 116 p.
269. **Minh Son Nguyen**. Oral health status and prevalence of temporo-mandibular disorders in 65–74-year-olds in Vietnam. Tartu, 2018, 182 p.
270. **Kristian Semjonov**. Development of pharmaceutical quench-cooled molten and melt-electrospun solid dispersions for poorly water-soluble indomethacin. Tartu, 2018, 125 p.
271. **Janne Tiigimäe-Saar**. Botulinum neurotoxin type A treatment for sialorrhea in central nervous system diseases. Tartu, 2018, 109 p.
272. **Veiko Vengerfeldt**. Apical periodontitis: prevalence and etiopathogenetic aspects. Tartu, 2018, 150 p.
273. **Rudolf Bichele**. TNF superfamily and AIRE at the crossroads of thymic differentiation and host protection against *Candida albicans* infection. Tartu, 2018, 153 p.
274. **Olga Tšuiko**. Unravelling Chromosomal Instability in Mammalian Pre-implantation Embryos Using Single-Cell Genomics. Tartu, 2018, 169 p.
275. **Kärt Kriisa**. Profile of acylcarnitines, inflammation and oxidative stress in first-episode psychosis before and after antipsychotic treatment. Tartu, 2018, 145 p.
276. **Xuan Dung Ho**. Characterization of the genomic profile of osteosarcoma. Tartu, 2018, 144 p.
277. **Karit Reinson**. New Diagnostic Methods for Early Detection of Inborn Errors of Metabolism in Estonia. Tartu, 2018, 201 p.
278. **Mari-Anne Vals**. Congenital N-glycosylation Disorders in Estonia. Tartu, 2019, 148 p.

279. **Liis Kadastik-Eerme**. Parkinson's disease in Estonia: epidemiology, quality of life, clinical characteristics and pharmacotherapy. Tartu, 2019, 202 p.
280. **Hedi Hunt**. Precision targeting of intraperitoneal tumors with peptide-guided nanocarriers. Tartu, 2019, 179 p.
281. **Rando Porosk**. The role of oxidative stress in Wolfram syndrome 1 and hypothermia. Tartu, 2019, 123 p.
282. **Ene-Ly Jõgeda**. The influence of coinfections and host genetic factor on the susceptibility to HIV infection among people who inject drugs. Tartu, 2019, 126 p.
283. **Kristel Ehala-Aleksejev**. The associations between body composition, obesity and obesity-related health and lifestyle conditions with male reproductive function. Tartu, 2019, 138 p.
284. **Aigar Ottas**. The metabolomic profiling of psoriasis, atopic dermatitis and atherosclerosis. Tartu, 2019, 136 p.
285. **Elmira Gurbanova**. Specific characteristics of tuberculosis in low default, but high multidrug–resistance prison setting. Tartu, 2019, 129 p.
286. **Van Thai Nguyeni**. The first study of the treatment outcomes of patients with cleft lip and palate in Central Vietnam. Tartu, 2019, 144 p.
287. **Maria Yakoreva**. Imprinting Disorders in Estonia. Tartu, 2019, 187 p.
288. **Kadri Rekker**. The putative role of microRNAs in endometriosis pathogenesis and potential in diagnostics. Tartu, 2019, 140 p.
289. **Ülle Võhma**. Association between personality traits, clinical characteristics and pharmacological treatment response in panic disorder. Tartu, 2019, 121 p.
290. **Aet Saar**. Acute myocardial infarction in Estonia 2001–2014: towards risk-based prevention and management. Tartu, 2019, 124 p.
291. **Toomas Toomsoo**. Transcranial brain sonography in the Estonian cohort of Parkinson's disease. Tartu, 2019, 114 p.
292. **Lidiia Zhytnik**. Inter- and intrafamilial diversity based on genotype and phenotype correlations of Osteogenesis Imperfecta. Tartu, 2019, 224 p.
293. **Pilleriin Soodla**. Newly HIV-infected people in Estonia: estimation of incidence and transmitted drug resistance. Tartu, 2019, 194 p.
294. **Kristiina Ojamaa**. Epidemiology of gynecological cancer in Estonia. Tartu, 2020, 133 p.
295. **Marianne Saard**. Modern Cognitive and Social Intervention Techniques in Paediatric Neurorehabilitation for Children with Acquired Brain Injury. Tartu, 2020, 168 p.
296. **Julia Maslovskaja**. The importance of DNA binding and DNA breaks for AIRE-mediated transcriptional activation. Tartu, 2020, 162 p.
297. **Natalia Lobanovskaya**. The role of PSA-NCAM in the survival of retinal ganglion cells. Tartu, 2020, 105 p.
298. **Madis Rahu**. Structure and blood supply of the postero-superior part of the shoulder joint capsule with implementation of surgical treatment after anterior traumatic dislocation. Tartu, 2020, 104 p.

299. **Helen Zirnask**. Luteinizing hormone (LH) receptor expression in the penis and its possible role in pathogenesis of erectile disturbances. Tartu, 2020, 87 p.
300. **Kadri Toome**. Homing peptides for targeting of brain diseases. Tartu, 2020, 152 p.
301. **Maarja Hallik**. Pharmacokinetics and pharmacodynamics of inotropic drugs in neonates. Tartu, 2020, 172 p.
302. **Raili Müller**. Cardiometabolic risk profile and body composition in early rheumatoid arthritis. Tartu, 2020, 133 p.
303. **Sergo Kasvandik**. The role of proteomic changes in endometrial cells – from the perspective of fertility and endometriosis. Tartu, 2020, 191 p.