Charles University

Second Faculty of Medicine

Doctoral study programme: Cellular Biology and Pathology

MUDr. Markéta Bloomfield

Monogenic susceptibility to infectious pathogens

Monogenně podmíněné vnímavosti k infekčním patogenům

Dissertation Thesis

Supervisor: prof. MUDr. Anna Šedivá, DSc.

Consultant: RNDr. Zuzana Paračková, Ph.D.

Prague, 2022

DECLARATION

Prohlášení

Prohlašuji, že jsem tuto práci vypracovala samostatně a že jsem řádně uvedla a citovala všechny použité prameny a literaturu. Tato práce ani žádné její části nebyly použity k získání jiného či stejného titulu.

Uděluji souhlas s trvalým uchováním elektronické verze této práce v databázi systému meziuniverzitního projektu Theses.cz za účelem soustavné kontroly podobnosti kvalifikačních prací.

V Praze, 30.09.2022

MUDr. Markéta Bloomfield

.....

ACKNOWLEDGEMENT

I would like to express my gratitude to the many people who have guided me through my training and professional carrier so far. Firstly, to my supervisors, particularly to Prof. MUDr. Anna Šedivá, DSc. and doc. MUDr. Hana Houšťková, CSc. Many thanks belong to my colleagues and friends in the Department of Immunology of University Hospital in Motol and in the Department of Pediatrics in Thomayer University Hospital, particularly, in respect to my academic work, to my consultant RNDr. Zuzana Paračková, Ph.D. Also, I would like to acknowledge the bravery of our patients and their families who made our research possible.

Finally, my deepest thanks belong to my family for their relentless mental and practical support.

ABSTRAKT (CZ)

Moderní přístupy ke studiu monogenních vrozených poruch imunity, podpořené v posledních dekádách bezprecedentním rozvojem genetických metod, odkrývají nové, dosud neprobádané funkční aspekty imunitního systému. Nemoci s nápadným klinickým fenotypem, leč víceméně normálními základními imunologickými nálezy, jako jsou poruchy vrozené či intrinsické imunity se selektivně zvýšenou náchylností k jedinému infekčnímu agens, poskytují vzácnou příležitost ke studiu interakcí imunitního systému člověka s patogeny. Tato práce se zaměřuje na imunopatologické, genetické a klinické aspekty takových onemocnění, konkrétně na chronickou mukokutánní kandidózu způsobenou hypermorfními (gain-of-function, GOF) mutacemi ve *STAT1* genu, které způsobují poruchy Th17 asociovaných imunitních mechanismů, a vrozenou náchylností k mykobakteriálním onemocněním (Mendelian susceptibility to mycobacterial diseases, MSMD) způsobenou poruchami signální dráhy IL-12, IL-23/IFNγ. Práce dále přispívá k objasnění role IL-6 signalizace v protistafylokokové imunitě a zabývá se novým onemocněním dětského věku PIMS-TS (Paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2), jako život ohrožujícím důsledkem imunopatologie spuštěné jediným konkrétním patogenem, který se s vysokou pravděpodobností vyvíjí v důsledku individuální, dosud neznámé genetické predispozice. Poznatky prezentované v této práci bylo možné v několika případech přenést přímo do klinické praxe, např. použití JAK inhibitorů u pacientů se STAT1 GOF a úpravu dávkování podle nově vyvinutého STAT fosfoflow protokolu, doporučení k očkování proti SARS-CoV-2 u STAT1 GOF pacientů, profylaxi a léčbu IFNγ u pacientů s AD parciálním deficitem IFNγR1, individuální terapeutická doporučení pro pacienta s unikátní kombinovanou poruchou IFNγ a NOD2 signalizace nebo identifikaci prediktorů závažnosti u PIMS-TS a doporučené terapeutické strategie u tohoto onemocnění.

Klíčová slova:

vrozené poruchy imunity, infekce, Candida, mycobacterium, SARS-CoV-2, IL-12, IL-23, IFNγ, MSMD, Th17, STAT1, IL-6, PIMS-TS, MIS-C

ABSTRACT (ENG)

The modern approach to studies of monogenic inborn errors of immunity, driven by unprecedented advances of genetic tools, opens vast undiscovered areas of immune system components and functions. In particular, the diseases with striking clinical phenotypes with normal or near normal baseline immunophenotype, such as disorders of innate and intrinsic immunity with susceptibility to single pathogen, provide a unique window into the host-pathogen interactions. This thesis covers various novel aspects of immunopathology, genetics and clinical facets behind some such diseases, namely chronic mucocutaneous candidiasis due to hypermorphic (gain-of-function, GOF) *STAT1* mutations, which hamper Th17-associated immune activities, and Mendelian susceptibility to mycobacterial diseases (MSMD) due to impairment of IL-12, IL-23/IFNγ signalling pathway. Moreover, it contributes to the mounting evidence that IL-6 signalling is non-redundant in anti-staphylococcal immunity. Finally, it explores the novel Paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) as a single pathogen-driven life-threatening immunopathology, which likely develops due to individual, yet unknown, genetic predisposition. The findings presented in this thesis were in several cases translated directly into the patients' clinical management, for example the use of JAK inhibitors in STAT1 GOF patients and the use of newly developed STAT phosphoflow protocol for dose adjustments, the recommendations on vaccination against SARS-CoV-2 in STAT1 GOF patients, the prophylaxis and treatment with IFNγ in patients with AD partial IFNγR1 deficiency, individualized therapeutic recommendation for a patient with unique combined impairment of IFNγ and NOD2 signalling, or the identification of severity predictors in PIMS-TS and its recommended management strategies.

Key words:

inborn errors of immunity, infections, Candida, mycobacterium, SARS-CoV-2, IL-12, IL-23, IFNγ, MSMD, Th17, STAT1, IL-6, PIMS-TS, MIS-C

LIST OF ABBREVIATIONS

ACT1	actin1
AD	autosomal dominant
AIDS	acquired immunodeficiency syndrome
AIRE	autoimmune regulator
APS1	autoimmune polyglandular syndrome type 1
AR	autosomal recessive
BAFF	B-cell activating factor
BAFFR	B-cell activating factor receptor
BCG	Bacillus Calmette-Guérin
BTK	Bruton tyrosine kinase
CARD9	caspase activation and recruitment domain-containing 9
CCR5	C-C chemokine receptor type 5
CGD	chronic granulomatous disease
CMC	chronic mucocutaneous candidiasis
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CXCL9/10	C-X-C motif chemokine ligand 9/10
CYBB	cytochrome B-245 beta chain
DARC	Duffy antigen and receptor f or chemokines
DC-SIGN	dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
dsRNA	double-stranded ribonucleic acid
EBMT	European society for blood and marrow transplantation
ESID	European society for immunodeficiency
EV	epidermodysplasia verruciformis
FUT2	fucosyltransferase 2
GAF	gamma-activating activating factor
GAS	gamma-activating activating sequences
GATA2	GATA-binding factor 2
GLDC	glycine decarboxylase
GOF	gain-of-function
GP130	glycoprotein 130
HIV	Human immunodeficiency virus
HLA	human leukocyte antigen
HPV	Human papillomavirus
HSCT	hematopoietic stem cell transplantation
HSE	Herpes simplex encephalitis
HSV	Herpes simplex
IDDA	immune deficiency and dysregulation activity
IEI	inborn error(s) of immunity
IFNα	interferon alpha
IFNβ	interferon beta
IFNγ	interferon gamma
IFNω	interferon omega
IFNλ	interferon lambda
TNF-α	tumor necrosis factor alpha
IFNAR1/2	interferon alpha receptor subunit 1/2
IFNG	interferon gamma
IFNGR	interferon gamma receptor
IgG, A, M	immunoglobulin G, A, M
IGRA	IFN gamma-release assay
IL	interleukin

IL1R	interleukin 1 receptor
IL6R	interleukin 6 receptor
IL12RB1/2	interleukin 12 receptor beta subunits 1/2
IL17F	interleukin 17F
IL17RA	interleukin 17 receptor A
IL17RC	interleukin 17 receptor C
IL23R	interleukin 23 receptor
IPEX	immunodysregulation-polyendocrinopathy-enteropathy X-linked syndrome
IRAK4	IL1R–associated kinase 1,4
IRF	interferon regulatory factor
ISG15	interferon-stimulated gene 15
ISGF3	interferon-stimulated gene factor 3
ISRE	interferon-stimulated response element
IUIS	International union of immunological societies
JAK	Janus kinase
JNK1	c-Jun N-terminal kinase 1
LPS	lipopolysaccharide
MDA5	melanoma differentiation-associated protein
MINCLE	macrophage inducible Ca 2+ -dependent lectin receptor
MIS-C	multisystem inflammatory syndrome in children
MSMD	Mendelian susceptibility to mycobacterial diseases
MyD88	myeloid differentiation primary response 88
NEMO	NF-κB essential modulator
NF- κB	nuclear factor kappa B
NK	natural killer
NLR	nod-like receptor
NLRP3	nod-, lrr- and pyrin domain-containing 3 protein
NOD2	nucleotide-binding oligomerization domain-containing protein 2
NTM	non-tuberculous mycobacteria
PAMPS	pathogen associated molecular patterns
PBMC	peripheral blood mononuclear cell
PIMS-TS	paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2
PRR	pattern recognition receptors
RANTES	regulated upon activation, normal T cell expressed and presumably secreted
RLR	Rig-like receptor
ROR	RAR-related orphan receptor
RORC	RAR-related orphan receptor C
RPSA	ribosomal protein SA
RORγ	RAR-related orphan receptor gamma
rt-PCR	real-time polymerase chain reaction
SAP	SLAM-associated protein
SLAM	signalling lymphocyte activation molecule
SARS-COV-2	severe acute respiratory syndrome coronavirus 2
SCID	severe combined immunodeficiency
SOCS-1	suppressor of cytokine signalling 1
SPB	surfactant protein B
SSPL2A	signal sequence peptidase-like 2a
STAT1,3	signal transducer and activator of transcription 1, 3
TB	tuberculosis
TBK1	TANK-binding kinase 1
TBX21	T-box transcription factor 21, T-bet
TGF-β	transforming growth factor beta
Th17	T helper 17 lymphocyte

TICAM	Toll-interleukin 1 receptor domain (TIR)-containing adaptor molecule
TLR	Toll-like receptor
TMC6/8	transmembrane channel-like 6/8
TRAF	TNF receptor- associated factor 1
TRAPS	TNF receptor-associated periodic syndrome
Treg	regulatory t cell
TRIF	TIR-domain-containing adapter-inducing interferon beta
TYK2	tyrosine kinase 2
TYR	tyrosine
XIAP	X-linked inhibitor of apoptosis protein
XLA	X-linked agammaglobulinemia
ZNF341	zinc finger protein 341
ZNFX1	zinc finger NFX1-type containing 1

TABLE OF CONTENTS

INTRODUCTION

1.1 INBORN ERRORS OF IMMUNITY

Inborn errors of immunity (IEI), or primary immunodeficiencies, represent a group of rare diseases driven by genetic defects, that profoundly affect the functions of immune system. The failure of human immune defences manifests characteristically as increased microbial susceptibility which results in frequent, chronic, often severe or even life-threatening infections. Given the organizational complexity and multilevel interactions of the human immune processes with the other microsystems, it is unsurprising that IEI also manifest with symptoms spanning broadly beyond infections. In fact, most patients with IEI also suffer from various degree of immune dysregulation arising either as a direct consequence of the genetic defect or, secondarily, during the recruitment of homeostatic regulatory mechanisms. Both instances may lead to abnormal mistargeted immunologic hyperreactivity presenting as allergic or autoimmune manifestations and may also result in failure of tumour immunosurveillance.

IEI are rare diseases. Their overall incidence varies geographically; in most European countries, including the Czech Republic, the incidence does not exceed 2-3/100 000 inhabitants. At the time of conception of this thesis, the Czech National IEI Register included approximately 1000 patient records. As of 2022, a total of 485 IEI were described, however a great deal of them is still represented by individual patients or kindreds only (Tangye S. et al., 2022). Although the first IEI, the Bruton agammaglobulinemia, was described 70 years ago (Bruton O.,1952), it wasn't until the turn of the millenium that the rapidly developing methods of genetic analyses enabled an overt boom of precision diagnostics in the field of IEI. The specific clinical and immunophenotypical clusteres became linked with genotypes and many crucial discoveries were made concerning the origin, development, purpose and regulation of various constituents of the immune system. Until nowadays, the investigations of patients with novel genetic defects presenting with unique phenotypes keep providing invaluable clues into the intricate immune molecular-cellular machinery and facilitate the development of precision therapeutic strategies aiming towards individually- tailored treatments. Based on the expanding knowledge, IEI are now classified into ten specific categories, although many disorders span phenotypically across more than one category. These are listed in Table 1. The most frequent IEI are antibody deficiencies, accounting for over 55% of all IEI (Durandy A. et al., 2013). The disorders discussed in this thesis belong to the group entitled Disorders of intrinsic and innate immunity, which are ultrarare, representing less than 2% of IEI.

Table 1. Phenotypical classification for IEI with examples of diseases, according to International Union of Immunological Societies (IUIS) (Tangye S. et al., 2022)

1.	Combined cellular and humoral immunodeficiencies	Severe combined immunodeficiency HyperIgM syndrome Reticular dysgenesis
2.	Syndromic immunodeficiencies	DiGeorge syndrome Wiskott-Aldrich syndrome HyperIgE syndrome
3.	Predominantly antibody immunodeficiencies	X-liked agammaglobulinemia Common variable immunodeficiency Selective IgA deficiency
4.	Diseases of immune dysregulation	IPEX syndrome Autoimmune lymphoproliferative syndromes Familial hemophagocytic lymphohistiocytoses
5.	Phagocytic disorders	Congenital neutropenia Chronic granulomatous disease Leukocyte adhesion deficiency
6.	**Defects of innate and intrinsic immunity**	Mendelian susceptibility to mycobacterial diseases Chronic mucocutaneous candidiasis Isolated congenital asplenia
7.	Autoinflammatory diseases	TRAPS Familial Mediterranean fever HyperIgD syndrome
8.	Complement deficiencies	Deficiencies of C5-C9 components (disseminated Neisseria predisposition) Hereditary angioedema Haemolytic-uremic syndrome
9.	Bone marrow failure	Fanconi anaemia Dyskeratosis congenita Mirage syndrome
10.	Phenocopies of immunodeficiencies	Autoantibodies against IFNγ Autoantibodies against IL-17/IL-22 Pulmonary alveolar proteinosis

1.2 INNATE, ADAPTIVE AND INTRINSIC IMMUNITY

Immune mechanisms are traditionally divided into the frontline innate and experience-shaped adaptive compartments. However, such oversimplified and rigorous segregation is grossly underappreciative of the complexity and interconnectivity of both immune mechanisms, as evidenced by the many disorders affecting primarily innate components which also disrupt the functionalities of adaptive mechanisms, and vice versa. Nevertheless, for scholarly purposes the classification provides a level of advantageous clarity. While the adaptive pool comprises of three cell types only, i.e., αβ T lymphocytes, γδ T lymphocytes, and B lymphocytes, the innate armament is much more diverse. It encompasses a three-tier defence system, which includes 1. mechanical/chemical barriers (such as skin, ciliary clearance, low gastric pH) 2. humoral factors [such as soluble factors in saliva, mucus or breast milk, complement system, acute phase reactants, and broad range of cytokines, particularly type I interferons alpha, beta and omega (IFNα, IFNβ, IFNω), type II interferon gamma (IFNγ) and type III interferon delta (IFNλ), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, IL-12, IL-12, IL-10, transforming growth factor beta (TGF-β)] and 3. a magnitude of innate immune cells with diverse functions and origin. These are either bone marrow-derived (neutrophil granulocytes, monocytes, macrophages, dendritic cells, natural killer cells, innate lymphoid cells) or of non-hematopoietic origin (e.g., epithelial and endothelial cells, keratinocytes, fibroblasts) (Medzhitov R. and Janeway C., 2000; Mogensen T., 2009).

Innate immunity orchestrates the first line of protection against pathogens, which is initiated directly upon the contact. Almost immediately, multiple soluble factors get engaged in a fight against viruses, bacteria, fungi or parasites. Within hours, various cellular signalling cascades are engaged chiefly by the ligand activation of molecular sensors called pattern recognition receptors (PRRs), such as Toll-like (TLR), NOD-like (NLR), RIG-like (RLR) receptors, double-stranded ribonucleic acid (dsRNA)-activated protein kinase receptors, C-type lectin receptors, and others. These are highly evolutionarily conserved receptors unique to microorganisms, which respond to various microbial components and products, called pathogen-associated molecular patterns (PAMPS). They include, for example, the bacterial lipopolysaccharides, lipoproteins, peptidoglycans, zymosan, mannan, flagellin or microbial nucleic acids (Li D. and Wu M., 2021; Mogensen T., 2009). Innate immune processes are principally always the same in nature and diverse only in the temporospatial aspects. Under physiologic conditions, they are short-spanned and tightly contra regulated to avoid excessive activation and mistargeted inflammation, which would inflict self-induced tissue damage and give rise to autoinflammatory disorders.

Although efficient and quick in action, innate immunity operates via non-specific antimicrobial tools only, as it bridges the time until the adaptive immunity gets involved, which is usually within days. Contrary to the innate, the adaptive mechanisms target non-self antigens with high selectivity and specificity, which is achieved by somatic genetic mutations. Moreover, ait is the main instrument of immune memory. Despite their varied roles, both subsystems are complementary and integrated, aiming for the most efficient, yet controlled host protection (Alberts B. and Johnson A., 2002).

In between the conventional innate and adaptive immune responses, another intracellular mechanism of antiviral protection has evolved, called "intrinsic" immunity. The term refers to constitutively expressed array of genes and their protein products, that are ready at all times to restrict viral replication immediately upon sensing of the pathogen. Much like the innate immunity with its interferon-dominated antiviral response, the intrinsic restriction factors also respond in the same manner upon each contact with the virus; on the other hand, similarly to adaptive immunity, the intrinsic immunity targets a specific virus or specific viral taxa to achieve their attenuation (Bieniasz P., 2004; Yan N. and Chen Z, 2012). Some of the most studied intrinsic restriction factors interfere with *Herpes simplex virus 1* (such as promyelocytic leukaemia protein-nuclear body constituent proteins, E3 SUMO ligases or DNA repair proteins) (Alandijany T., 2019), *Human immunodeficiency virus* (such as Tripartite motif-containing protein 5 alpha, Apolipoprotein B mRNA editing enzyme, catalytic subunit 3G) (Stremlau M. et al., 2004; Sheehy A. et al., 2002) or *Cytomegalovirus* (such as Death-associated protein 6) (Saffert R. and Kalejta R., 2006).

1.3 THE GENETIC THEORY OF HUMAN INFECTIONS

With the identification of microorganisms as the agents of various human illnesses, most of the infectious diseases' severity was for some time attributed to the pathogen-related factors, such as the infectious dose, virulence, etc. The first suggestion that the outcome of individual host-pathogen interaction is to some extend heritable came from the 1930' observations of familial clustering of both rare and common infections, and from studies of subjects with variable (that is asymptomatic vs. symptomatic) course of infections (Nicolle C., 1937). By the turn of the 20th century, the molecular genetic methods have uncovered genetic backgrounds of several "conventional" IEI (Ochs H. and Hitzig W., 2012; Casanova J. L. et al., 2005; Notarangelo L. et al., 2006). Through these, much was discovered about the human immune system physiology. A paradigm was set in place, that increased infectious susceptibility segregates either in a Mendelian pattern of inheritance (typically recessive, with complete penetrance), or in a polygenic trait (theoretical). Regardless of

the genetic cause, the failure of a particular immune mechanism would result in multiple infectious phenotypes, i.e., infections with broad spectrum or certain characteristic groups of microbes. As such, the disrupted development of B lymphocytes and, consequently, the antibody production, increases the risk of infections with encapsulated bacteria; congenital defects of phagocytes predispose to severe fungal infections and infections with intracellular pathogens; defects of T cells impair defences against viruses, bacteria, fungi and parasites. In parallel, a new concept of the fundamental role of pathogens in shaping the genetic background of human anti-microbial defences throughout the evolution was suggested (Casanova J. L. and Abel L., 2004; Picard C et al., 2006). It was largely derived from descriptions of severe/fatal infections due to singular pathogen or a very narrow spectrum of microbes in otherwise healthy subjects. It was also supported by observation of peculiar clinical resistance to otherwise highly infectious pathogens, frequently with obvious intrafamilial segregation. Many of such rare phenotypes have now been associated with their respective genotypes which may, indeed, follow Mendelian traits (autosomal dominant, AD; autosomal recessive, AR; or X-linked), and are referred to as "nonconventional" IEI (Casanova J. L. and Abel L., 2005). They are, in main, caused by germline mutations in genes encoding components of innate immune mechanisms and often inflict much broader phenotypes of immune dysregulatory syndromes. Thus, in addition to the two main accepted notions, i.e., that 1) single-gene variant = vulnerability to multiple pathogens and 2) multiple genes variants = vulnerability to one or more pathogens (susceptibility to common infection is due to the combined effect of multiple genes, the "polygenic" trait), a third mechanism was uncovered, where 3) single-gene variant = vulnerability to single pathogen (Casanova J. L. et al., 2005). Originally, a complete penetrance was assumed for single gene – single pathogen IEI, however in later identified kindreds incomplete penetrance traits were also noted. Furthermore, based on the complex segregation analyses and linkage studies another mechanism of "major gene" was proposed, suggesting that 4) only one mutated gene causes the single pathogen susceptibility/resistance, but other genes, epigenetic or environmental factors interfere with the gene expression to such extend that it may or may not manifest (thus accounting for the phenomenon of incomplete penetrance in the mutation carriers) (Chapman H. and Hill A., 2012) (Table 2).

Table 2. The mechanisms involved in genetic theory of infectious diseases in humans with examples (extended, based on Darrason M., 2013)

Genetic mechanism	Specifics	Examples	Affected gene(s)
MONOGENIC MENDELIAN SUSCEPTIBILITY TO INFECTIONS BY MULTIPLE PATHOGENS	Single gene Complete penetrance	Severe combined immunodeficiency	*IL2RG*
		X-linked agammaglobulinemia	*BTK*
		Congenital asplenia	*RPSA*
		Chronic granulomatous disease	*CYBB*
MONOGENIC MENDELIAN SUSCEPTIBILITY TO SINGLE PATHOGEN/ SINGLE GROUP OF MICROBES	Single gene High penetrance (but incomplete penetrance also described)	Epidermodysplasia verruciformis	*TMC6/8*
		HSV encephalitis	*TLR3*
		Susceptibility to atypical mycobacteria	*IFNGR*
POLYGENIC SUSCEPTIBILITY TO SINGLE/MULTIPLE PATHOGENS	Multiple genes Low penetrance	HLA-associated infections (tuberculosis, Dengue fever, hepatitis B, C, HIV, HPV)	
MAJOR GENE SUSCEPTIBILITY/RESISTANCE	Single major gene High/ intermediate penetrance	Resistance to *Plasmodium vivax* (malaria)	*DARC*
		Severe tuberculosis in adults	*8q12-q13 region*

These four genetic mechanisms are not by far mutually exclusive, but rather create a genetic continuum. Combined with the pathogen-related factors, such overlapping model neatly explains the inter-individual variability in response to pathogens via a unified, compellingly interactionist model (Figure 1). It is highly likely, that future discoveries will further expand and shape this current dogma.

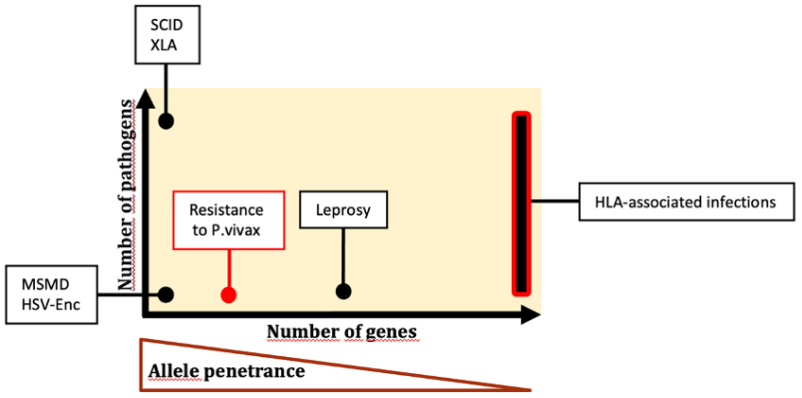


Figure 1. A model of genetic continuum of human infectious diseases with examples of respective diseases (modified from Alcaïs et al., 2009)

Black = susceptibility, red = resistance. HSV-Enc = HSV encephalitis; MSMD = Mendelian susceptibility to infectious diseases; P. vivax = *Plasmodium vivax*; SCID = Severe combined immunodeficiency; HLA = Human leukocyte antigen; XLA = X-linked agammaglobulinemia

1.3.1 Inborn resistance to specific pathogens

Resistance to specific virulent pathogens is one of the most intriguing phenomena in human immunogenetics. It describes why some individuals do not suffer from a penetrant disease despite being exposed to a pathogen which is otherwise highly infectious to others. These rare experiments of nature create a paradigm shift, in which the wild-type genotype confers an infection susceptibility risk, while the mutant alleles are protective.

The currently known examples of such single-gene microbial resistance are all explained by the lack of the microbe's point of entry into the host cells, which prevents the pathogen's replication and spread. On cellular level, the underlying germline mutations knock out the genes which encode integral parts of surface receptors on the cells targeted by the pathogen for invasion. Thus, the absence of chemokine receptor Duffy antigen and receptor for chemokines (DARC) on erythrocytes prevents the cellular invasion of *Plasmodium vivax,* the cause of malaria (Tournamille C. et al., 1995), the absence of C-C chemokine receptor type 5 (CCR5) on T cells averts the entry of *Human immunodeficiency virus (HIV)1* into T cells preventing AIDS (Acquired Immunodeficiency Syndrome) (Liu R. et al., 1996; Arenzana-Seisdedos F. and Parmentier M., 2006), the loss of fucosyltransferase 2 (FUT2) receptor on enterocytes protects from norovirus enteritis (Lindesmith L. et al., 2003; Thorven M et al., 2005), and the lack of P antigen on erythrocytes convey natural resistance to parvoviral erythema infectiosum (Brown et al., 1994). While these mutations are in principle deleterious on DNA level, they provide a distinct biologic advantage as these individuals are otherwise healthy and don't seem to have impaired immune functions in general. It is, therefore, easy to imagine that such disease-protective mutations, likely originally incidental, have been (and will be) the subjects of positive evolutionary selection, providing a population advantage under the continuous microbial pressure. On the other hand, it is conceivable that such benefit may come at a cost, perhaps in the inability to fend off other, yet unknown pathogens.

1.3.2 Inborn susceptibility to specific pathogens

Spearheaded by the teams of Jean-Laurent Casanova, Laurent Abel, and Alexandre Alcaïs, genetic variants rendering carriers vulnerable to single type, or a narrow spectrum of pathogens were extensively studied since the 1990'. The research aimed to explore the biological background of severe courses of primoinfections or recurrent reinfections by single/single type of organism in otherwise seemingly immunocompetent individuals.

Generally, these diseases share several hallmark features, which represent diagnostic clues:

- Severe/prolonged/treatment-refractory course of primary infection with the pathogen (that would usually not pertain such severe symptoms)
- Recurrent/persistent infections with the same pathogen despite adequate treatment
- Unincreased susceptibility to other microbes
- Normal/near normal basic immunologic parameters, (i.e., undisturbed lymphocyte and neutrophil counts, normal lymphocyte proliferation ability to non-specific stimuli, normal immunoglobulin IgG, IgA, IgM levels and IgG subclasses, normal granulocyte oxidative burst)
- Familial occurrence of the symptoms or consanguinity

Table 3 enlists (non-exclusively) IEI which are known to underlie a highly selective microbial vulnerability. For illustrative purposes, the following account portraits some of these diseases in more detail. Two entities, the Mendelian susceptibility to mycobacterial diseases (MSMD) and monogenic chronic mucocutaneous candidiasis (CMC), will be discussed separately in greater detail, as they represent the main area of the author's doctoral focus and the centerpoint of this thesis.

Table 3. IEI with selective susceptibility to a specific pathogen conferred by single-gene mutations

Disease	Pathogen	Genetic defects
Mendelian susceptibility to mycobacterial diseaeses	Non-tuberculous mycobacteria	IL-12/23 - IFNγ circuit
Chronic mucocutaneous candidiasis	Candida spp.	IL-17 signaling
Staphylococcal infections	Staphylococcus aureus	IL-6/IL6R/gp130
Invasive dermatophytosis	Dermathophytus spp.	CARD9
Invasive pneumococcal infection	Streptococcus pneumoniae	IRAK4, MyD88, NEMO
Tuberculosis	Mycobacterium tuberculosis	IL12Rβ1
Herpes simplex encephalitis	Herpes simplex	TLR3, T, TRAF, UNC93B
Epidermodysplasia verruciformis	Human papillomavirus	TMC6, TMC8
Severe influenza	Influenza virus	IRF7
Meningococcemia, meningococcal meningitis	Neisseria meningitidis	C5-C9
Whipple's disease	Tropheryma whipplei	IRF4
Severe Ebstein-Baar virus infection	Ebstein-Baar virus	SAP/SLAM, XIAP
Severe COVID-19	SARS-CoV-2	Type I IFN components, TLR7

1.4 MONOGENIC INBORN ERRORS OF IMMUNITY WITH SELECTIVE SUSCEPTIBILITY TO SINGLE/NARROW SPECTRUM OF PATHOGENS

1.4.1 Epidermodysplasia verruciformis

Epidermodysplasia verruciformis (EV) is one of the most intriguing human infectious disease described for the first time in 1922 (Lewandowsky F. and Lutz W., 1922), thus de facto representing the very first reported IEI, preceding even the X-linked agammaglobulinemia. This extremely rare, autosomal recessive dermatosis is caused by persistent human papillomavirus (HPV) infection, specifically by skin-specific EV-associated oncogenic β-HPV. The patients suffer from extensive growth of cutaneous warts, often of bizarre appearance resembling the bark of a tree, which gave the disease its other name "the treeman syndrome" (Figure 2). Most patients develop non-melanoma skin cancer by thirty years of age. Its monogenic background wasn't discovered until 2002 (Ramoz N. et al., 2002); over 50% of EV cases are caused by inactivating mutations affecting genes for transmembrane channel-like 6 (TMC6) and transmembrane channel-like 8 (TMC8) protein. Neither the physiologic function of these proteins, nor their role in HPV restriction in keratinocytes has been explained yet, however their involvement in zinc metabolism has been suggested (de Jong S. et al., 2018).

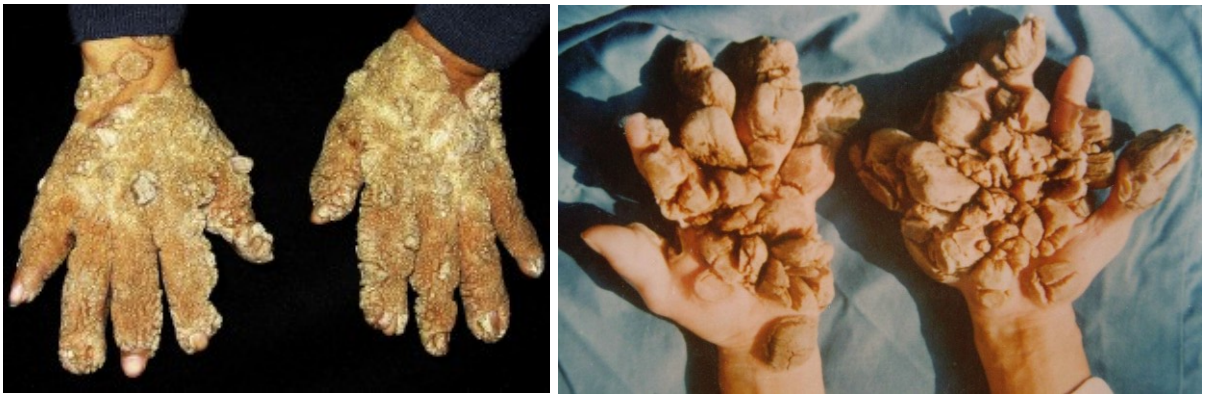


Figure 2. Epidermodysplasia verruciformis due to *TMC6/TMC8* mutations (Liu W. and Ma M., 2020; Fang F. et al., 2008)

1.4.2 Herpes simplex encephalitis

Infections with *Herpes simplex virus* (HSV) are amongst the most common infections in humans. HSV is a neuro- and epitheliotropic virus with the ability of life-long latent persistence in neurons. Primary infection with HSV1 occurs usually in infancy and is most typically

asymptomatic/subclinical, or manifests as painful, yet self-limiting mucocutaneous affliction, called aphthous gingivostomatitis. Reactivations of the virus result in infection of epithelial cells at the neuro-epithelial junction, characteristically presenting as cutaneous or mucosal vesicular lesions (Arduino P. and Porter P., 2008). In rare cases, the primary infection takes on a devastating course, causing HSV encephalitis (HSE) (Figure 3A). This striking phenotype has been, in some cases, explained by mutations in gene encoding components of toll-like receptor 3 (TLR3) signalling pathway (*TLR3, TRIF, TRAF3, UNC93B1, TBK1, IRF3*) (Zhang S.-Y. and Casanova J.L., 2015; Andersen L. et al., 2015) (Figure 3B). The impairment of TLR3 downstream signalling results in aberrant type I and type III IFN production, which prevents efficient viral clearance in neurons and oligodendrocytes. Curiously, the virus does not spread beyond the brain tissue as the TLR3-mediated HSV1 control mechanism seems to be redundant in other cell types (such as leukocytes, keratinocytes and fibroblasts). In fact, despite being intrinsically vulnerable to HSV1, these patients show a remarkable absence of mucocutaneous infections.

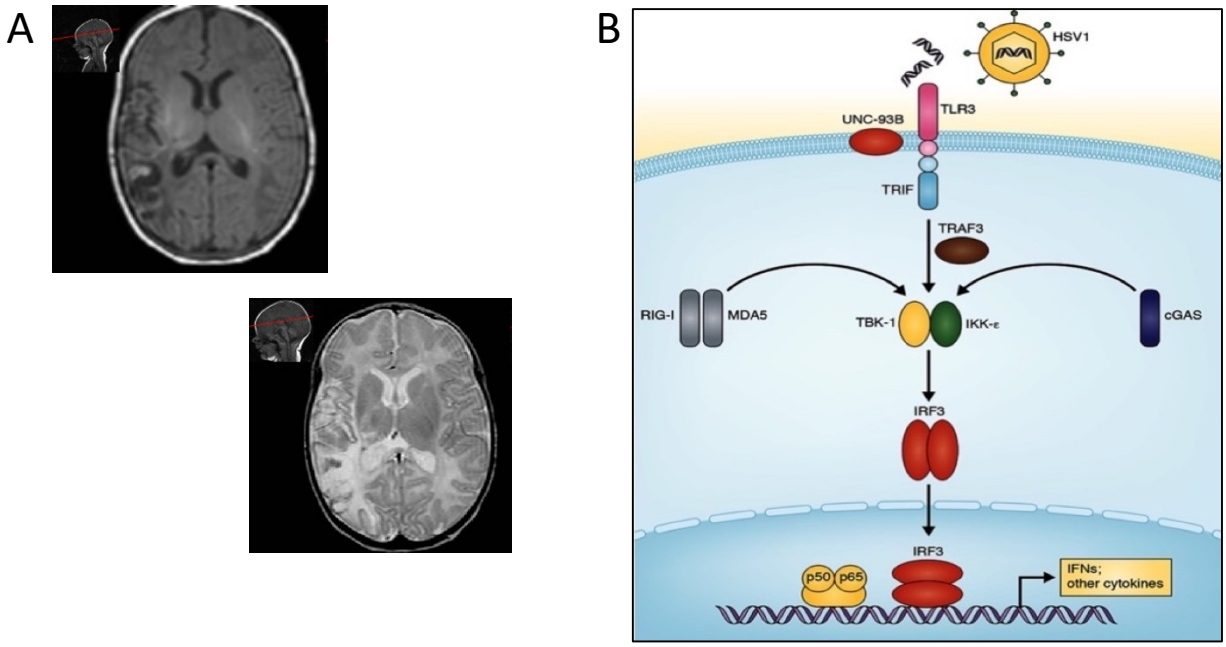


Figure 3. A) Brain magnetic resonance imaged of four-month-old infant with *Herpes simplex* encephalitis (T1- and T2-wieghted sequences; archives of the author) B) TLR3 signalling pathway showing the six known proteins encoded by HSV-1 Mendelian susceptibility genes (Zhang S.-Y. and Casanova J.L., 2015)

1.4.3 Severe/fatal influenza

A family of single-stranded RNA influenza viruses underlies the annual seasonal, as well as the irregular epidemic/pandemic human infections (World Health Organization 2018). In the majority of patients, influenza manifests as a self-limiting respiratory infection with mild-to-moderate symptoms. However, in some patients it may take on more serious course requiring hospital admission and ventilation support. This interindividual variability is determined by individual risk factors, such as pre-existing pulmonary, cardiovascular, neurologic co-morbidities, age, obesity, pregnancy or previous exposure to influenza (Abadom T. et al., 2016; Eski A et al., 2019; McCullers J., 2014), as well as the virulence of the influenza strain and the size of the infectious inoculum. The individual host genetic background is also an important facet (Clohisey S. and Baillie J., 2019)(Figure 4).

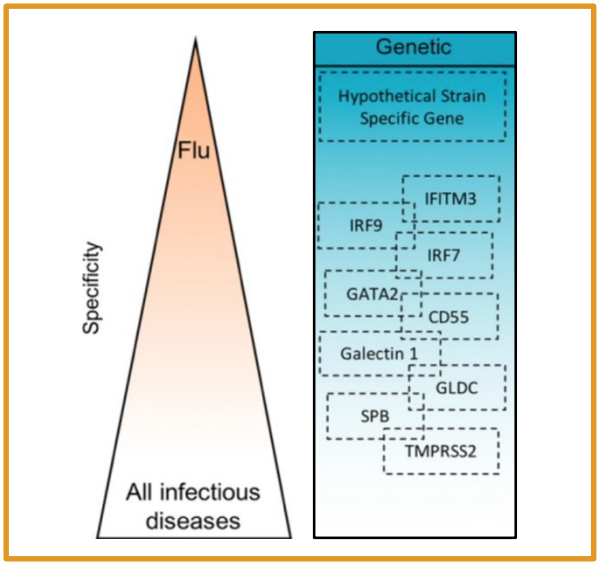


Figure 4. Illustration of genetic spectrum of human infectious susceptibility exemplified by susceptibility genes for influenza A (Flu) infections (adapted from Clohisey S. and Baillie J., 2019)

Comparing flu-susceptible population to control group, several genetic variants and polymorphisms have been associated with increased risk of severe influenza pneumonitis, affecting proteins involved in viral restriction control, interferon responses, inflammation/immune signalling or surfactant composition, such as Interferon-induced transmembrane protein 3, Transmembrane protease serine 2, TNF-α, IL-1, IL-6, IL-17, IL-28, or Pulmonary surfactant-associated proteins (Clohisey S. and Baillie J., 2019).

In very rare cases, otherwise healthy patients experience influenza as critical illness, typically as an acute respiratory distress syndrome or life-threatening encephalitis (Glaser C. et al., 2012). These patients were presumed to have disturbed amplification or response to interferons, and, correspondingly, individually documented kindreds were found to harbour AR or AD mutations in genes encoding interferon regulatory factors (IRF7 and IRF9) (Ciancanelli et al., 2015; Hernandez et al., 2018). Moreover, defects of genes encoding TLR3 protein (Lim et al. 2019) and GATA2 transcription factor (Sologuren et al., 2018) have been identified as causative for the influenza-specific failure of intrinsic immune responses. Recently, mutations in *DBR1,* an RNA lariat debranching enzyme, were shown to predispose to increased neurotropic sensitivity to very narrow spectrum of pathogens, i.e., influenza, HSV1 and norovirus (Zhang S. Y. et al., 2018). The interesting overlap of HSV1 neuron-specific susceptibility with influenza pneumocyte-specific susceptibility, conveyed by TLR3 deficiencies, leaves much to be explained. It is altogether obscure, why defects in such pleiotropic and ubiquitous innate mechanisms as interferon signalling pathways should account for the very narrow or even singular pathogen susceptibility, rather than an overall increased viral sensitivity. Perhaps the pathogen-specific reliance on activation of various IFN–dependent and IFN-independent signalling mechanisms might vary according to the cell/tissue type.

1.4.4 Severe pyogenic infections

In some patients with severe course of invasive bacterial infections (i.e., sepsis, meningitis, arthritis, osteomyelitis, severe pneumonia, or deep abscesses) by *Streptococcus pneumoniae spp., Staphylococcus aureus* and *Pseudomonas aeruginosa*, but with unincreased vulnerability to other pathogens and normal basic immune parameters, mutations in genes encoding components of proximal PRR-mediated canonical nuclear factor-kappa B (NF-κB)-dependent pathway were discovered (Courtois and Smahi., 2006; Ku et al., 2007; Picard et al., 2011). These include proteins involved in transduction of signal from TLR (other than TLR3) and most IL-1 receptors, namely MyD88 (Myeloid differentiation primary response gene 88), IRAK1 a IRAK4 (IL1R–associated kinase 1,4) or NEMO (essential modulator of NF-kB) (Figure 5). NF-kB is a ubiquitous protein complex with transcription factor function, that facilitates a broad spectrum of intracellular signalling resulting in upregulation and modulation of inflammatory responses (Picard et al. ,2003). In particular, biallelic loss-of-function mutations of *MyD88, IRAK4* and hemizygous *NEMO* mutations confer a narrow susceptibility to invasive pneumococcal diseases (C.-L. Ku, Picard, et al. 2007; Picard et al., 2003). Given the impaired upregulation of inflammatory molecules, these severe

infections are accompanied by surprisingly low increase of plasma C-reactive protein (CRP) and its inducer IL-6 concentrations. Also, the clinical signs of systemic inflammation are much less pronounced, for example only low fever is present at the beginning of infections due to IL-1 hypoproduction. Interestingly, in a proportion of the patients, the infectious susceptibility appears to be age-dependent, i.e., decreases with aging.

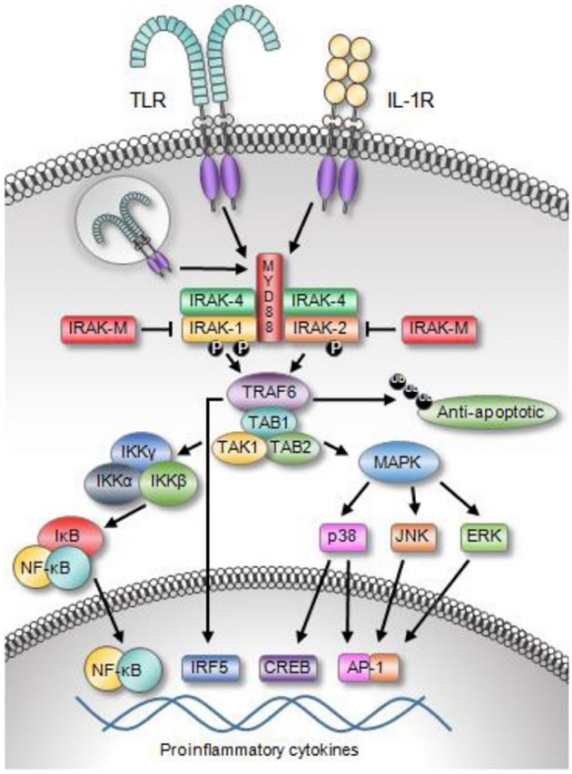


Figure 5. TLR, IL-1R family members signalling pathways and the pathway of NF-kB protein complex assembly (Jain et al. 2014)

1.4.5 Recurrent/severe staphylococcal infections

Staphylococcus aureus in an encapsulated gram-positive, facultative human pathogen. It is, however, also a common commensal of upper airways, detectable in up to 30% of healthy people's nares (Krismer et al., 2017). The pathogen-host interaction is of particularly complex nature and several IEI are hallmarked by increased susceptibility to *Staphylococcus aureus;* for instance, antibody and complement deficiencies, disorders of phagocytic killing, congenital asplenia, defects in distal components of canonical NF-kB pathway, or HyperIgE syndromes predispose individuals to Staphylococcal infections. Some even confer a risk of severe organ infections or sepsis. These

patients, however, also suffer from infections by array of other bacterial, fungal and viral taxa. On the other hand, the prominent vulnerability to *Staphylococcus aureus* in patients with AD HyperIgE syndrome, caused by loss-of-function mutations in signal transducer and activator of transcription (STAT) 3, implies that the functional integrity of IL-6/STAT3 signalling cascade is particularly important in the anti-staphylococcal innate immune defence (Park and Liu, 2020). IL-6 is a pleiotropic cytokine produced in response to danger signals, which participates in various biologic processes, including stimulation of acute phase responses, haematopoiesis and oncogenesis (Tanaka, Narazaki, and Kishimoto 2014) (Figure 6). Recently, sporadic patients with genetic loss of proteins involved in IL-6/gp130/IL6R/STAT3 signalling pathway (other than AD STAT3 loss-of-function mutations), specifically IL-6 receptor deficiency, biallelic mutation in *IL6ST* encoding the gp130 co-receptor, and missense mutations in *ZNF341* encoding a zinc finger transcription factor, were reported to confer selectively impeded resistance to *Staphylococcus aureus* (Spencer et al., 2019; Béziat et al., 2018; Frey-Jakobs et al., 2018). These findings were supported by individual reports of staphylococcal infections in rare patients with autoantibodies against IL-6 (Puel et al., 2008; Nanki et al., 2013), and by increased risk of staphylococcal infections in patients with rheumatologic diseases treated with IL-6 signalling blocking compounds (Nguyen et al. 2013).

Of note, an important clinical aspect of IL-6/STAT3 defects is the failure to mount an efficient acute phase response mirrored by diminished ability to increase serum CRP levels, as CRP is induced by IL-6 via STAT3.

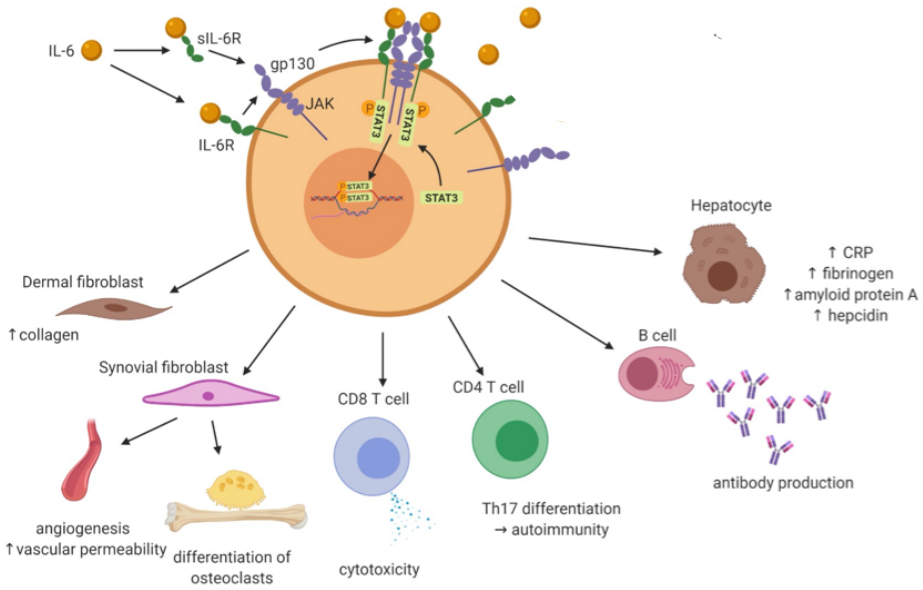


Figure 6. IL-6 signal transduction and its pleiotropic effect on upregulation of various biologic processes, including inflammatory mechanisms (inspired by Tanaka et al., 2011)

1.4.6 Meningococcaemia, meningococcal meningitis

The gram-negative encapsulated *Neisseria meningitidis* (the meningococcus) is the main cause of bacterial meningitis and septicaemia globally. In most carriers, it remains a harmless colonizer of the nasopharynx (in up to 10% of adolescents) and seldomly causes an invasive disease. In symptomatic cases, two epidemiological peaks typically occur; in infants >1 year of age and in adolescents and young adults between 15-25 years of age (Lewis and Ram, 2014). The individual vulnerability was puzzling until early observations that complement deficient people are at heightened risk of *Neisseria* infections (Figueroa and Densen, 1991).

The complement is a heavily regulated autocatalytic multienzyme system composed of 30+ proteins. It is activated in a stepwise manner by various exogenous and endogenous stimuli via the classic, alternative and lectin pathways, which converge in the C3 component. Distally from C3, the precisely orchestrated cleavage and interaction of terminal components C5-C9 result in the formation of a pore-shaped membrane attack complex with major bactericidal activity (Lewis and Ram, 2014; Hadders et al., 2012) (Figure 7). Patients with proximal component deficiencies are prone to infections by an array of pyogenic bacteria, as well as autoimmune features. Interestingly, studies of otherwise healthy survivors of severe or recurrent meningococcal infections identified genetic background in some patients, precluding functional deficiencies of factors C5-9 and properdin, the co-activator of alternative complement pathway. (Agarwal et al., 2010; Nürnberger et al., 1989; Kojima et al., 1998; Gianella-Borradori et al., 1988). Thus, deficiencies of terminal complement components underlie selective susceptibility to invasive meningococcal disease.

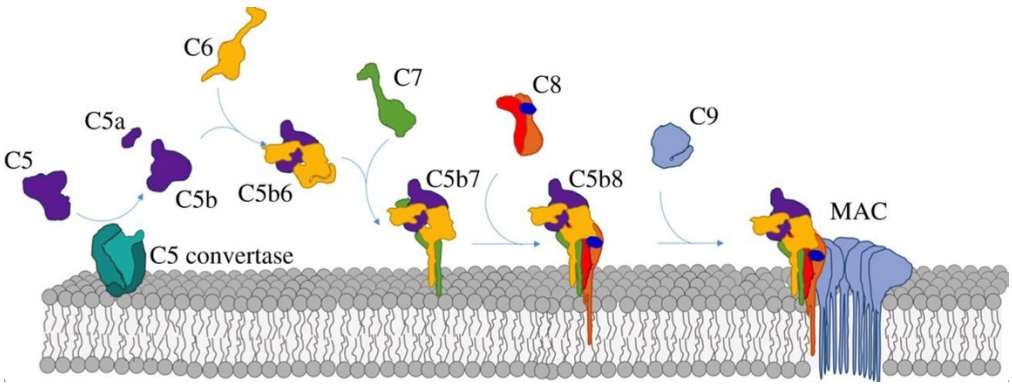


Figure 7. The generation of membrane attack complex from distal complement components (Hadders et al., 2012)

1.4.7 Whipple's disease

Originally described in 1907 as a non-infectious intestinal autoinflammatory disease by H. Whipple, the causative agent, gram-positive bacillus *Tropheryma whipplei,* was cultured only in 2000 (Raoult et al., 2000). The eponymous Whipple's disease usually presents in later decades of life (50-60 years of age) and takes on a chronic course, manifesting with intestinal and systemic symptoms (Braubach et al., 2017). Curiously, chronic asymptomatic carriage of *Tropheryma whipplei* is common in the general population. Recently, in a French study, IRF4 haploinsufficiency was shown to underlie severe Whipple's disease with incomplete penetrance. IRF4 is expressed exclusively in immune cells (macrophages, dendritic cells, T and B lymphocytes) and takes part in regulation of leukocytes' differentiation and activation (Nam and Lim, 2016). As such, AD mutations in *IRF4* represent another single-gene cause of a severe course of primary infection in otherwise healthy subjects (Guérin et al., 2018).

1.4.8 Severe COVID-19

Most recently, the global occurrence of the novel human beta-coronavirus SARS-CoV-2 provided a unique opportunity to study the host-pathogen interaction in a naïve population on both individual and populational level. As soon as the interindividual variability of the disease, called COVID-19, became apparent, various risk factors were suggested to associate with its severity, such as age, obesity, diabetes, hypertension, coagulation dysfunctions, inflammation disorders, and others (Wolff et al., 2021). In parallel, corresponding to the human genetic theory of infections, the team around Professor Casanova formulated a hypothesis that in some patients, particularly in young, healthy adults, life-threatening COVID-19 may be due to an unknown monogenic IEI. Engaging the healthcare providers and researchers from all around the world in the COVID Human Genetic Effort project, several susceptibility loci known to govern TLR3–, IRF7– and TLR7-dependent type I IFN immunity were found to underlie autosomal recessive, autosomal dominant or X-linked deficiencies. Together, these defects (in *TLR3, TICAM, TBK1, UNC93B1, IRF3, IRF7, IFNAR1, IFNAR2* and *TLR7*) account for approximately 5% of severe COVID-19 infections in patients with no prior severe infections (Q. Zhang et al., 2020; Asano et al., 2021) (Figure 8A).

At the same time, supporting evidence of the non-redundant role of type I IFN signalling in protection against severe COVID-19 was delivered by the detection of high titres of neutralizing autoantibodies against IFNα and IFNω in about 10% of patients with severe COVID-19 pneumonia

(Bastard et al., 2020) (Figure 8B). Contrasting with severe COVID-19 patients with inborn genetic defects, these autoantibodies were predominantly found in older patients (> 60 years of age).

Remarkably, four months after the first COVID-19 cases were reported, a new life-threatening paediatric illness appeared in the high incidence communities, which was clearly temporally associated with SARS-CoV-2 infection and thus became known as PIMS-TS (Paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2), or later MIS-C (Multisystem inflammatory syndrome in children) (Riphagen et al., 2020). This hyperinflammatory syndrome affects otherwise healthy children and resembles other childhood entities with overt inflammatory responses, such as toxic shock syndrome, macrophage activating syndrome, or the Kawasaki disease, a vasculitis of unknown aetiology with weak seasonal association with viral infections (Dietz et al., 2017). As such, a novel theory that rare IEI altering the immune response to SARS-CoV-2 may underlie the pathogenesis of MIS-C (and, by extension, the Kawasaki and other diseases) in some children (Sancho-Shimizu and Brodin, 2021) arose and is currently being tested. As a proof of principle, deleterious defects in *XIAP* (encoding X-linked inhibitor of apoptosis), *CYBB* (encoding beta subunit of cytochrome b-245) and haploinsufficiency of SOCS1 (suppressor of cytokine signalling 1) have already been shown to convey a genetic risk of MIS-C (Lee et al., 2020; Chou et al., 2021).

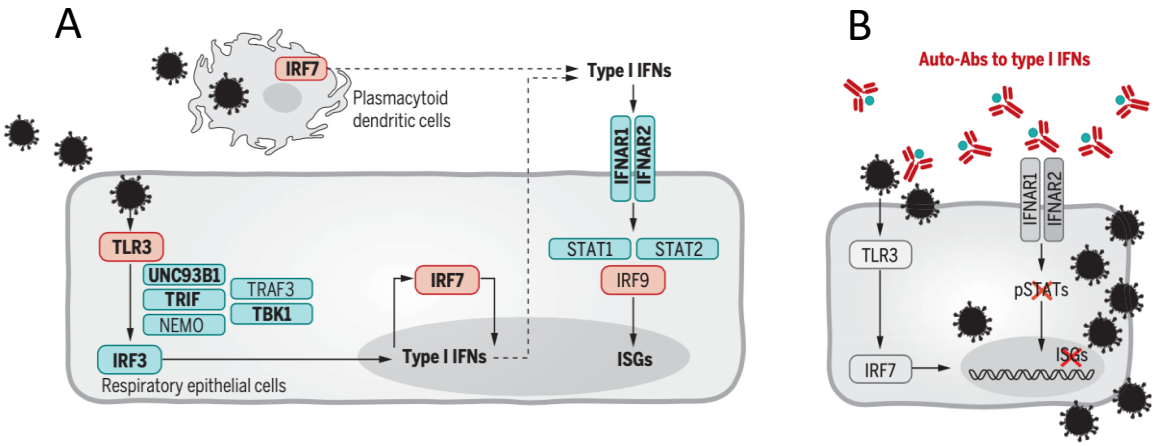


Figure 8. A) Inborn errors of TLR3- and IRF7-mediated type I interferon immunity in severe COVID-19. In bold = proteins encoded by COVID-19 susceptibility genes (Q. Zhang et al., 2020) B) The effects of type I interferon autoantibodies, found in patients with severe COVID-19 (Bastard et al., 2020)

1.4.9 Mendelian susceptibility to mycobacterial diseases (MSMD)

1.4.9.1 Mycobacteriacae and mycobacterial infections in humans

Mycobacteriacae is a multidiverse genus encompassing species that have infected humans since prehistoric era. In fact, tuberculosis (TB), caused by *Mycobacterium (M.) tuberculosis,* is the oldest known human contagious disease, documented by the detection of the bacterial DNA in the human fossils dating 9000 years back (Hershkovitz et al., 2008). Fascinating indirect evidence suggests its possible presence in *Homo erectus* 500,000 years ago (Kappelman et al., 2008). The genus includes over 170 species, which are traditionally divided into three groups, according to their clinical significance: 1. the strict pathogens, the TB-causing *M. tuberculosis,* the leprosy-causing *M. leprae,* and the bovine *M. bovis* 2. the non-tuberculous, or atypical mycobacteria (NTM), such as *M. avium complex, M. abscessus complex, M. fortuitum, M. marinum,* and *M. ulcerans* 3. the rare saprophytic organisms, such as *M. smegmatis* (Khandelwal and Dubey, 2020). *M. tuberculosis* is a highly contagious agent, claiming over 1.5 million deaths per year worldwide (Koch and Mizrahi, 2018). On the contrary, NTM are opportunistic, weakly virulent pathogens, found ubiquitously in soil, water reservoirs and vegetation, rarely causing human infections (Porvaznik et al., 2017).

If clinically penetrant, the infections with NTM most commonly manifest as pulmonary infections in adults (accounting for up to 85% of all adult NTM infections), lymphadenopathy in children (most commonly cervical lymphadenitis by *M. avium complex*), or by cutaneous lesions (Zhou et al., 2022; López-Varela et al., 2015; Lamb and Dawn, 2014; Jones et al., 2018). Risk factors for pulmonary NTM include the presence of underlying lung disease, low body-mass index and various secondary immunodeficient states affecting the cellular compartment per se, such HIV infection, treatment with TNF-α targeting agents, anti-IL-12/23 monoclonal antibodies or corticosteroids. Various IEI also predispose to infections with NTM, such as severe combined immunodeficiency (SCID), HyperIgM syndrome, chronic granulomatous disease (CGD) and MSMD (discussed in detail below) (Henkle and Winthrop, 2015; Reichenbach et al., 2001). These IEI also represent increased risk of disseminated or severe localized infections, which appear in approximately in 15% of all NTM infections (Swenson et al., 2018). On the other hand, traumatic or surgical wounds soiled with contaminated materials increase the risk of cutaneous NTM even in immunocompetent patients (Lamb and Dawn, 2014).

The diagnosis of NTM infection relies on a high degree of clinical suspicion. The gold diagnostic standard is culture-positivity, which is, however, tricky to obtain due to weeks-long incubation period requiring special rich media (e.g., Löwenstein-Jensen, Middlebrook 7H11-mycobactin

medium) and rapid bacterial overgrowth (Nicolas et al., 2010). Other direct methods of pathogen detection utilize (immuno)histochemical acid-fast staining (Ziehl-Neelsen, auramine-rhodamine fluorescent stain), immunohistochemistry with specific fluorescent labels, and modern real-time polymerase chain reaction (rt-PCR) assays (Choi et al., 2012). Characteristic histopathologic pattern of mycobacterial infection is the necrotising epithelioid granuloma, composed of activated macrophages, multinucleated giant cells of Langhan´s type, and $CD4^+$ T lymphocytes in the microenvironment governed mainly by IL-12 and IFNγ cytokines (Shah et al., 2017). The assessment of antigen-specific cellular immune recall, such as IFNγ-release assays (IGRA) and intradermal skin testing with purified protein derivate (Mantoux test) or aviary sensitin may be diagnostically utilized (Hermansen et al., 2014; Bilbo et al., 2018). Moreover, indirect supportive indices of NTM presence may be the negative results of conventional microbiological studies and usually unspecific laboratory parameters with only mildly elevated inflammatory markers.

Treatment of NTM is notoriously precarious, owing mainly to a high natural resistance of NTM to first-line anti-TB drugs and the necessity of prolonged treatment (Wi, 2019).

Interestingly, the incidence of NTM has been reported to be increasing in the past decades, particularly in countries with decreasing TB incidence (K. L. Chin et al., 2020), which is likely due to improving access to diagnostic resources, and possibly due to the discontinuation of population-wide TB vaccination with attenuated *Bacillus Calmette-Guérin* (BCG), which is presumed to provide some level of protection against NTM (Dolezalova and Gopfertova, 2021) (Figure 9). This represents a challenge for the clinicians, particularly for the paediatric immunologist, because opportunistic NTM infections represent the classic red flags for several IEI.

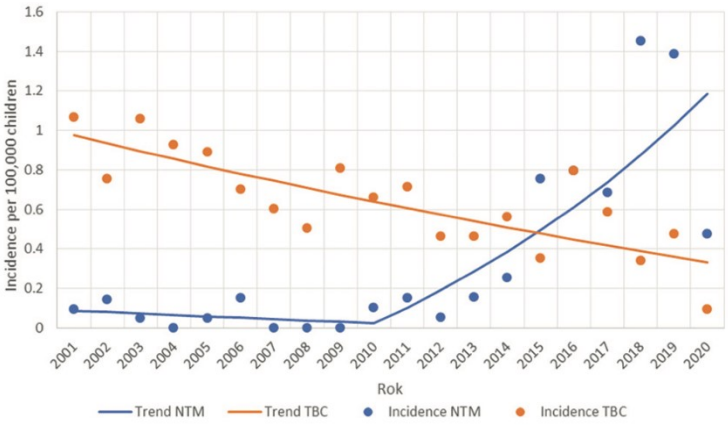


Figure 9. Cumulative incidence of infections with *M. tuberculosis* (causing tuberculosis, TBC) and non-tuberculous mycobacteria (NTM) in the Czech paediatric population in the past two decades (the population-wide vaccination with BCG was discontinued in 2010) (Dolezalova and Gopfertova, 2021)

1.4.9.2 Antimycobacterial immunity - overview

Mycobacteria are intracellular pathogens capable of infecting any human tissue. The antimycobacterial immunity is a multifaceted complex of immediate and long-term response actions, which may result in complete clearance, latency, or progression of the infection. The first barrier counteracting the host invasion by mycobacteria are the epithelial cells. Beyond the provision of physical obstacle, they also recognize mycobacterial PAMPS via TLR, nucleotide-binding oligomerization domain-containing protein 2 (NOD2), Dectin-1, c-type lectin and mannose receptors (Ferguson and Schlesinger, 2000). Consequently, they produce various antimicrobial peptides (particularly LL-37, β-defensin 2 and hepcidin) and immune attractants (e.g., TNF-α, granulocyte-macrophage colony-stimulating factor, RANTES, C-X-C motif chemokine ligand 9 and 10) (Li et al., 2012). These mediators draw an array of cells, i.e., phagocytes, neutrophils, professional antigen presenting cells and lymphocytes, to the site of infection (Griffith et al., 2014), where the mainstay of antimycobacterial defence rests upon the cooperation of innate and adaptive immune mechanisms. This is predominantly ensured by the IL-12, IL-23/ IFNγ-mediated communication pathway between mononuclear phagocytes (i.e., macrophages, monocytes and dendritic cells) and type 1 helper T cells/NK cells (Figure 10). IL-12, IL-23 and IFNγ are the backbone activators of proinflammatory signals and activities in these cells, augmenting processes such as antigen presentation, intracellular killing via production of reactive oxygen species, microbicidal nitric oxide or phagosome-lysosome fusion and lysosomal degradation, and paracrine secretion of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-8 (Jalaledin et al., 2021). The histologic correlate of such complex orchestration is the sealing of mycobacteria within the granulomas patrolled by phagocytes and walled by the rim of $CD4^+$T cells (Russell, 2007).

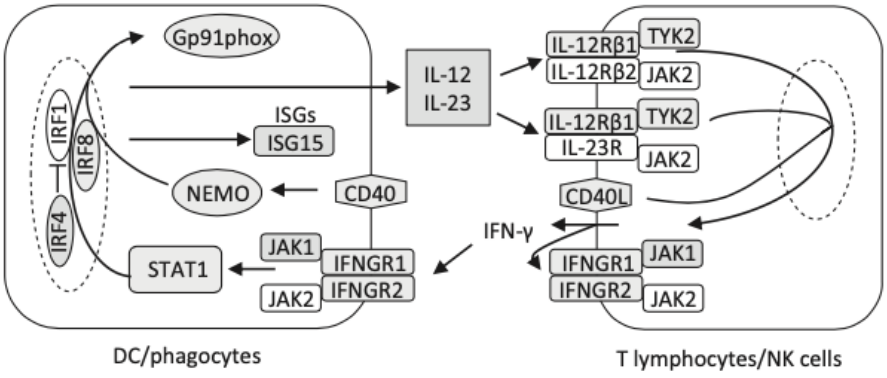


Figure 10. Schematic representation of components of IL-12, IL-23/IFNγ signal transduction between mononuclear phagocytes (dendritic cells - DC, monocytes, macrophages) on the left and T and NK (natural killer) cells on the right (Bucciol et al., 2019)

The humoral immunity, i.e., the complement and antibody-mediated responses, facilitating opsonization for enhanced phagocytosis or direct killing of mycobacteria by complement-mediated lysis or antibody-dependent cytotoxic activity of NK cells, were somewhat disregarded in the past as to their contribution to the clearance of intracellular mycobacteria (Bohlson et al., 2001; Achkar et al., 2015). Although still controversial, recent evidence points to specific antimycobacterial antibody efficacy, which may differ according to isotype and body compartment (Zimmermann et al., 2016).

Mycobacteria have developed a surprisingly broad range of strategies of immune evasion and survival (Fraga et al., 2018). It is likely that the human heritable antimycobacterial defence machinery evolved to such a complex system of interconnected redundant and non-redundant mechanisms thanks to the millennia-long host-pathogen interactions. Consequently, several genetic hosts factors are now known to confer variable levels of susceptibility to mycobacteria.

1.4.9.3 Genetic host factors of mycobacterial susceptibility

On one hand, genome-wide association studies and linkage analysis identified susceptibility loci of variable strength of association with mycobacterioses (Thye et al., 2010, 2012; Curtis et al., 2015; Sveinbjornsson et al., 2016; Zheng et al., 2018). On the other hand, classic single-gene IEI with numeric or functional defects of T cells, dendritic cells and aberrant intramacrophageal killing confer increased susceptibility to NTM, amongst various other pathogens (Duncan and Hambleton, 2014). The third class of genetic susceptibility is represented by a group of rare monogenic inborn errors of intrinsic immunity, affecting the IL-12, IL-23/IFNγ circuit, which underlie a highly selective susceptibility to weakly virulent NTM and attenuated BCG vaccinal substrains, the Mendelian susceptibility to mycobacterial diseases (MSMD). To date, over 450 patients have been reported globally to harbour mutations in one of the 18 known MSMD-associated genes involved in IFNγ production (i.e., *IFNG, IL12, IL12RB1, IL12RB2, IL23R, ISG15, RORC, SSPL2A*), in response to IFNγ (i.e., *IFNGR1, IFNGR2, STAT1, JAK1, TYK2, CYBB*), or in both (*IRF8, NEMO*) (Bustamante 2020; Bustamante et al. 2014) (Figure 11). Recently, mutations in *ZNFX1* and *TBX21* were also added to the genetic aetiology of MSMD (Noma et al., 2022).

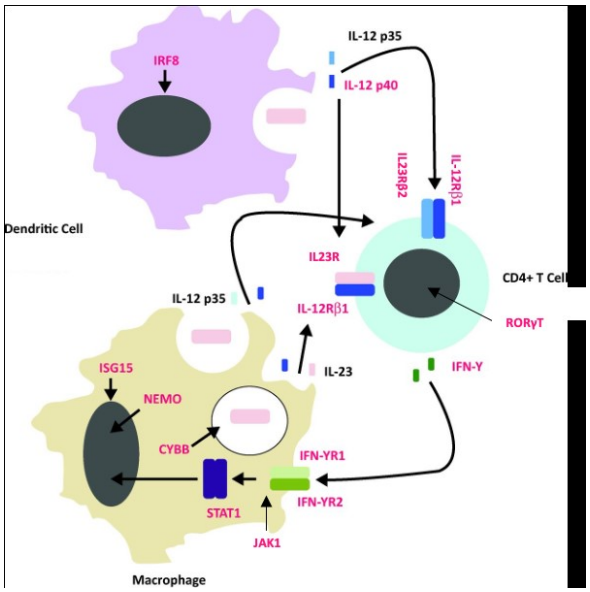


Figure 11. Proteins affected by single-gene mutations underlying Mendelian susceptibility to mycobacterial diseases. Reported defects are highlighted in red (updated, based on Duncan and Hambleton, 2014)

Finally, an interesting aetiology of adult-onset MSMD phenocopy was reported – the presence of IFNγ autoantibodies (Browne et al., 2012; Kampmann et al., 2005; Lee et al., 2013). However, the pathophysiologic mechanism underlying the genesis of these autoantibodies is yet to be explained.

Unsurprisingly, patients harbouring biallelic, otherwise populationally rare variants are often recruited from consanguineous families. The frequency of reported MSMD genetic aetiology is shown in Figure 12.

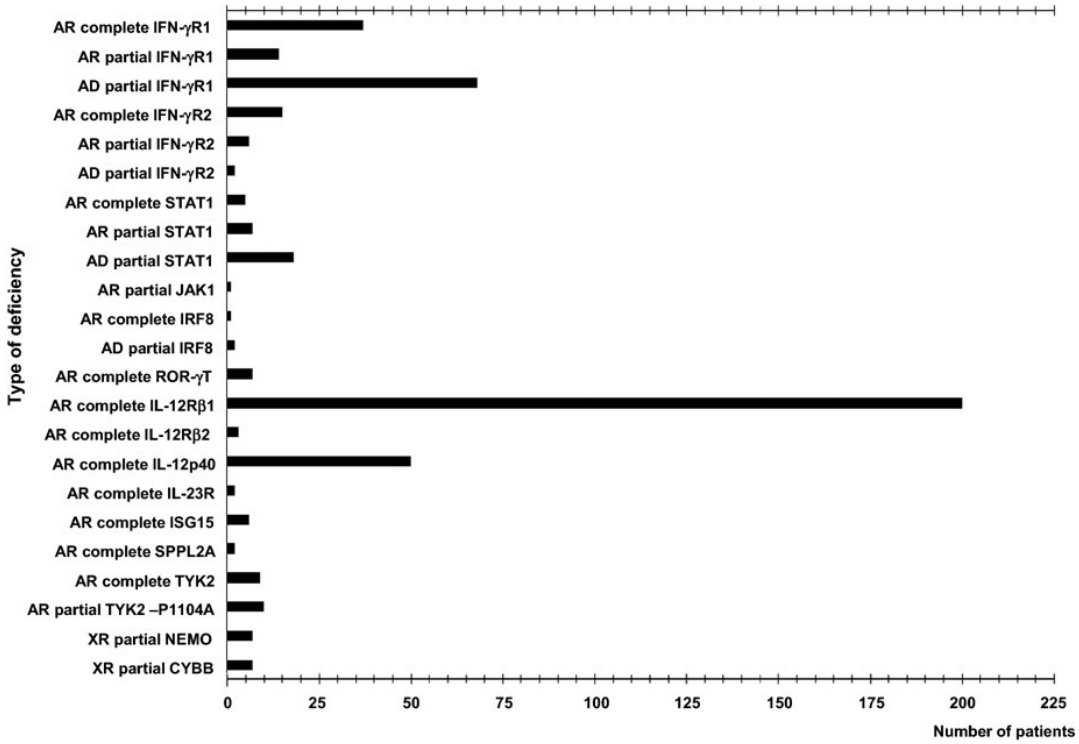


Figure 12. The total number of individuals with each MSMD aetiology as reported globally in 2020 (Bustamante, 2020)

1.4.9.4 Clinical features of MSMD

MSMD typically manifests in childhood, particularly in infants who receive BCG vaccine, but may present later in adolescence or adulthood. Although some patients also suffer from non-typhoid salmonellae or viruses (particularly the *Herpesviridae* family), the opportunistic mycobacterial vulnerability dominates the clinical phenotype. The symptoms range from adverse reactions to BCG vaccination, such as BCGitis – the inoculation site-limited infection associated with regional lymphadenopathy, to life-threatening organ/disseminated mycobacteriosis, often treatment-refractory or recurrent (Taur et al., 2021). The severity and course of the disease is strongly governed by the genotype; some mutations underlie a partial loss of signal transduction within the IL-12, IL-23/IFNγ pathway (e.g., AD *IFNGR1* or *STAT1* mutations) and display variable expressivity and incomplete penetrance, others are responsible for complete block (e.g., AR *IFGR1* and *IL12RB* mutations) with invariantly fatal prognosis (Bustamante, 2020; Boisson-Dupuis et al., 2012; Kerner et al., 2020). As with many other rare diseases, the genotype-phenotype correlations are being reinforced with every reported kindred.

Despite the clinically prominent immune failure, the routinely examined parameters of humoral and cellular immune functions are more or less normal in majority of MSMD patients, which, paradoxically, represents a red flag for this IEI. In patients harbouring CYBB mutations the failure of generation of oxidative burst may be detected (Khan et al., 2016), and in patients with RORC mutations the development of Th17 lymphocytes is hampered (Okada et al., 2015).

Patients with MSMD and active mycobacterial infections are treated with prolonged courses of combinations of anti-TB drugs, preferably according to the antibiotic susceptibility of the particular strain. Because of the high antibiotic resistance of NTM, second-and third-line anti-TB drugs are usually used. Patients with (at least residually) preserved functional capacity to transduce IFNγ signal benefit from treatment with recombinant IFNγ, while disorders with completely abolished signalling ability are not thus ameliorable. In these patients, hematopoietic stem cell transplantation (HSCT) may represent a potentially curative strategy, albeit extremely risky in the settings of active mycobacterial infection (Chantrain et al., 2006; Olbrich et al., 2015) and due to a hight risk of graft rejection, likely associated with the persistently increased serum level of IFNγ (Rottman et al., 2008).

The establishment of genetic diagnosis of MSMD enables genetic counselling for the affected families, however the individual predicament may be challenging to elucidate, given the phenomena of incomplete penetrance and variable expressivity in many of the reported MSMD kindreds.

For illustrative purposes, the following text describes two genetic aetiologies of MSMD, which were so far identified in Czech patients. These patients were the once involved in the research projects of this author. The other MSMD aetiologies have been either reviewed or described individually in comprehensive details elsewhere (please, refer to the reference list).

1.4.9.5 Autosomal dominant partial IFNγR1 (AD IFNγR1) deficiency

AD IFNγR1 deficiency was described in 1996 as the bona-fide first IEI with a monogenic selective infectious susceptibility (Jouanguy et al., 1996). By 2020, more than 50 unrelated families had been described worldwide, of which five patients were diagnosed in the Czech Republic. Most often, the heterozygous mutation is located in the hotspot encoding the cytoplasmic domain of the R1 subunit of the IFNγ receptor (Jouanguy et al., 1999; Bustamante et al., 2014). These mutations exert a so-called dominant negative effect - the receptor subunit encoded by the mutated allele is both non-functional and, at the same time, accumulates on the cell surface, thus interfering with the normal function of receptors encoded by the wild-type allele. As a result, the signalling is significantly reduced. The disease shows broad clinical heterogeneity and incomplete penetrance. The overall mean age of onset is 13.4 years, depending largely on whether BCG vaccine is administered (Dorman et al., 2004). In addition to localized or disseminated atypical mycobacteriosis, patients are also at greater risk of invasive non-typhoid *Salmonellae.* Moreover, a characteristic feature of IFNγR1 deficiency is mycobacterial multifocal osteomyelitis. This affinity for bone tissue is not satisfactorily explained, although enhanced IFNγ -dependent inhibition of osteoclast formation was demonstrated recently to impair the osteoclastogenesis in IFNγR1 and STAT1 deficiencies (Tsumura et al., 2022). The routinely tested cellular and humoral immune parameters are normal in majority of patients, unless skewed secondarily by the active mycobacterial (or other) infection. Importantly, the clinical utility of non-direct tests (i.e., Mantoux test or IFNγ-release assays) for the detection of mycobacteria may be hampered by the impaired cellular responsiveness, therefore cultures and direct detection methods (such as PCR, immunofluorescence) are more reliable. The ability to form specific granulomas is highly individual, reflecting each patient's ability to produce or respond to IFNγ (Lammas et al., 2002).

The diagnosis is established by genetic testing. The functional integrity of the IL-12, IL-23/IFNγ circuit can also be examined by assessing the cytokine secretory response to stimulation with BCG, IL-12 or IFNγ (Feinberg et al., 2004), STAT phosphorylation and IFNγ-inducible gene expression assays (Bloomfield et al., 2018) in response to IFNγ. Moreover, the accumulation of IFNγR is detectable by flow cytometry and, in the author's experience, represents a quick screening tool for

IFNγR1 deficiency. Excepting the genetic evaluation, the aforementioned tests are not routinely available in the clinical setting and require individually tailored research approach.

The treatment of patients with AD IFNγR1 constitutes of multidrug regimens with anti-TB drugs administered for months/years and, if indicated, regular subcutaneous administration of IFNγ (Kerner et al., 2020). Surgical debridement of the sites of infections is sometimes required. Long-term prophylaxis with anti-TB drugs is also utilized in patients with recurrent mycobacterial infections. If appropriately managed, patients typically survive well into adulthood (Dorman et al., 2004).

The first literary accounts of probable MSMD in former Czechoslovakia date back to 1959 and 1977, reporting two cousins with disseminated BCG and salmonellosis, therefore resembling the symptoms of AD IFNγR1 deficiency (Dvořáček et al., 1959; Dolečková et al., 1977). The first Czech patient with genetic aetiology of MSMD, the AD IFNγR1 deficiency, was identified in 2013 and reported by the author of this thesis (Bloomfield et al., 2016) (Figure 13). Since then, three other patients and one asymptomatic carrier were diagnosed in the Czech Republic.

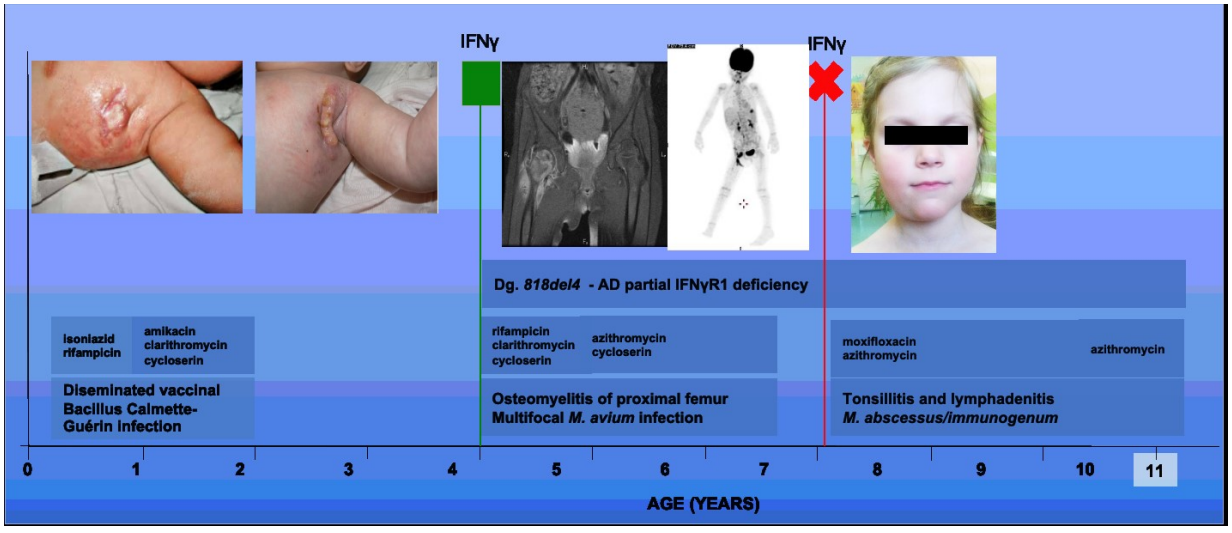


Figure 13. The timeline of clinical manifestations of MSMD and treatment of a Czech child with autosomal dominant partial IFNγR1 deficiency. Green square, red cross = initiation and discontinuation of IFNγ treatment, respectively (expanded from Bloomfield et al., 2016).

1.4.9.6 Autosomal dominant partial STAT1 deficiency

STAT1 is an important prototypical cytokine-responsive transcription factor, transducing signals from interferons and other factors involved in immune responses against intracellular pathogens and viruses (Schindler et al., 2007; Pestka et al., 2004). In the context of antimycobacterial immunity, STAT1 mediates IFNγ signalling after its ligation to IFNγR via the Janus kinase (JAK)-STAT pathway (similarly to IL-27 signalling). The phosphorylated STAT1 homodimerizes into gamma-activating factor (GAF) which translocates to nucleus to bind to the gamma-activating sequence (GAS) within promoters of IFNγ-inducible genes (Decker et al., 1997). The STAT1-mediated antiviral signalling is orchestrated differently. During the type I interferon-driven recruitment of STAT1, phosphorylated STAT1 forms a heterodimer with STAT2 and IRF9, known as interferon-stimulated gene factor 3 (ISGF3), which is translocated to nucleus and binds the IFN-stimulated response element (ISRE) to upregulate the IFNα- and IFN-β target genes (Horvath, 2000; Bluyssen and Levy, 1997) (Figure 14).

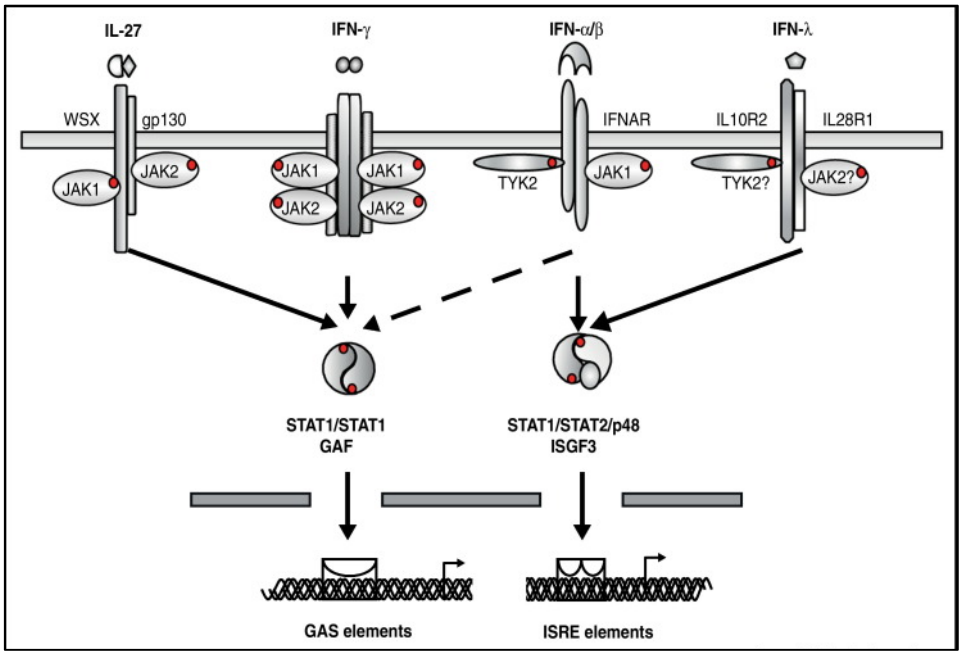


Figure 14. The dual role of Signal transducer and activator of transcription 1 in type I, II, III interferons and IL-27 signalling (Boisson-Dupuis et al., 2012)

Four distinct phenotypes of STAT1 genetic variants are now recognized: AR complete STAT1 deficiency, AR partial STAT1 deficiency, AD partial STAT1 deficiency, and AD STAT1 gain-of-function (Mizoguchi and Okada, 2021) (Figure 12). The first three of these convey MSMD phenotype

with or without increased susceptibility to other intracellular bacteria and viruses, depending on whether the IFNγ-activated responses are disturbed only, or if the IFNα/β responsiveness is also corrupted (Averbuch et al., 2011; Boisson-Dupuis et al., 2012). The AD partial STAT1 mutations have been identified in less than 50 patients worldwide (Zhang et al., 2021). The molecular mechanisms of decreased STAT1 signalling is mutation-specific and causes either decreased STAT1 phosphorylation, reduced DNA-binding capacity of GAF, its defective nuclear transport, or both the latter (Liu et al., 2022; Dupuis et al., 2001; Tsumura et al., 2012). Similar to the AD partial IFNγR1 deficiency, the mutations exert a dominant-negative effect on wild-type STAT1 and incomplete penetrance and variable expressivity is common (Bhattad et al., 2021). The disease usually manifests in childhood or adolescence, displaying clinical signs similar to AD IFNγR1, i.e., BCGitis in vaccine recipients (Figure 15A), NTM infections of variable extend (Figure 15B), as well as recurrent or multifocal osteomyelitis (Averbuch et al., 2011; Liu et al., 2022; Hirata et al., 2013).

AD partial STAT1 deficiency confers selective predisposition to mycobacterial infections, due to its impaired GAF-, but not ISRE-mediated responses to interferons. This is in strong contrast to AR complete STAT1 deficiency, which renders patients vulnerable to viruses besides NTM (mainly *Herpesviridae*) (Boisson-Dupuis et al., 2012). Such disparity is likely owing to the quantitative representation of STAT1 in homodimeric GAF and heterodimeric (ISGF3) STAT1 complexes.

The diagnosis is nowadays established primarily by genetic analysis, and may be supported by STAT phosphorylation assays (which, however, only detect mutations affecting STAT1 phosphorylation), evaluation of GAS-inducible genes expression in response to IFNγ (Bloomfield et al., 2018) or stimulation assays with BCG, IL-12 or IFNγ (Feinberg et al. 2004). The same limitations for the ascertainment of mycobacteria apply as for the AD partial IFNγR1 deficiency, including the restricted response in IFNγ-release assays and possibly restricted capability to generate granulomas. The patients are treated with prolonged multidrug anti-TB regimens and may also benefit from adjuvant IFNγ. HSCT is not recommended due to the usually milder phenotype (Zhang et al., 2021).

The first Czech patient with AD partial STAT1 deficiency was identified by the author of this thesis in cooperation with prof. Bustamante (Laboratory of Human Genetics of Infectious Diseases, Paris) in 2016. Since then, 1 other patient and 4 asymptomatic carriers were found (Figure 15).

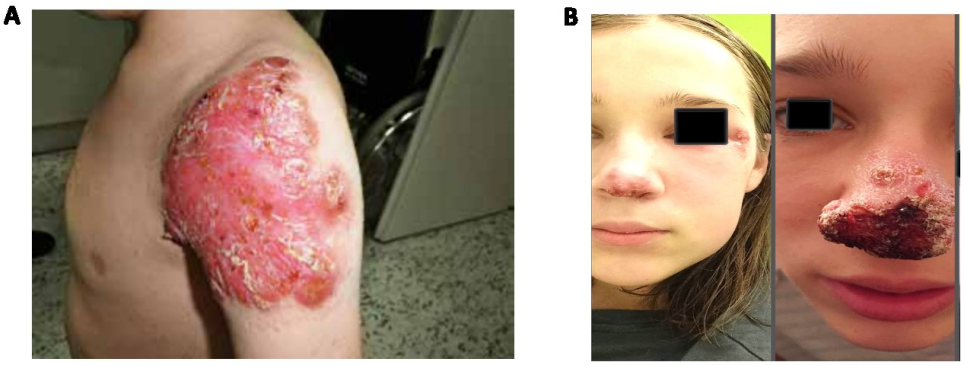


Figure 15. A) Reactivation of vaccinal *Bacillus Calmette-Guérin* (BCG) at the site of its inoculation, presenting as lupus vulgaris in otherwise healthy 6-year-old male with AD partial STAT1 deficiency. B) *Mycobacterium marinum* infection of nose in a in otherwise healthy 16-year-old female with AD partial STAT1 deficiency (Dolezalova et al., 2022)

1.4.10 Chronic mucocutaneous candidiasis (CMC)

1.4.10.1 Candida species and Candida infections in humans

Candida, a fungal genus of *Ascomycetes* containing over 200 species, is a white asporogenous dimorphic yeast capable of hyphae formation. It includes seven medically important species, e.g., *C. albicans*, *C. tropicalis*, *C. parapsilosis C. glabrata*, of which *C. albicans* accounts for up to 80% of all *Candida* strains isolated from infected patients. *Candida* is a common human commensal of the healthy and one of the most prominent opportunistic pathogen affecting the immunocompromised (Chin et al., 2016), as exemplified by the high rates of intensive-care units bloodstream infections (Schelenz, 2008; Muderris et al., 2020). *Candida* species harmlessly colonize cutaneous and mucosal sites of the body of 30% of healthy individuals, particularly the skin, mouth, gut and genitals. However, if the immune barriers are breached, the yeast may invade locally and cause surface-limited candidiasis, manifesting as skin lesions, mucositis of the mouth, onychomycosis of the nails, or gastrointestinal (e.g., oesophagitis) and respiratory (e.g., laryngotracheitis) mucositis. The chronic mucocutaneous candidiasis is then defined as persistent or recurrent skin, nail, and mucosal infection (Liu et al., 2011). In most severe cases, it may spread into any organ and cause disseminated parenchymal candidiasis and candidemia (Plantinga et al., 2012).

The risk factors of penetrant *Candida* infections are many, both inborn and acquired, often iatrogenic. Surface-limited mucocutaneous infections are more common in individuals with compromised skin/mucosal barriers, artificial dentures, increased skin contact with water,

systemic antibiotic therapy, secondary immune suppression or IEI affecting the Th17 lymphocytes functionality (López-Martínez, 2010; Millsop and Fazel, 2016). Risk factors for invasive *Candida* infections include comorbidities and medical interventions, such as immunosuppression due to any secondary cause (e.g., systemic steroid administration), the use of broad spectrum antibiotics, indwelling central venous catheters, mechanical ventilation, renal replacement therapy, parenteral nutrition, diabetes mellitus, malnutrition, intensive care unit stay, as well as several primary IEI, particularly the numeric and functional defects of neutrophils, (Thomas-Rüddel et al., 2022).

The diagnosis of infection with *Candida* rests upon culture results which also warrants the most appropriate selection of antifungal treatment according to the antibiotic resistance of the yeast strain. The diagnosis of systemic infections may be supported by serologic tests detecting 1,3 beta-D-glucan, mannan or anti-mannan antibodies (Piskorska et al., 2014; Bloos et al., 2018), both 1,3 beta-D-glucan and mannan being the integral *Candida* wall polysaccharide (Domer, 1989).

The therapeutic repertoire for candida infections is relatively broad, utilizing compounds such as azoles, amphotericin B and echinocandins (Glöckner et al., 2009). However, a looming current threat lies within the increase of antifungal drug resistant strains of *Candida* (Shawn et al. 2012; Lockhart et al., 2017).

1.4.10.2 Anti-Candida immunity - overview

The human anti-*Candida* immunity constitutes of several integrated layers of protection which share the key features across various tissues yet demonstrate certain tissue-specific differences, modulated by whether the yeast or hyphae morphogenetic forms are being fended off (Figure 16).

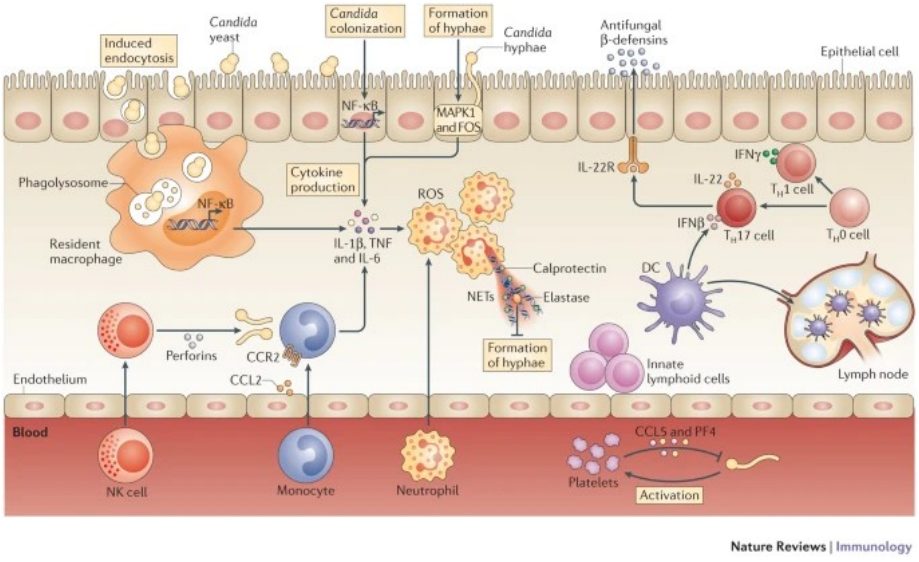


Figure 16. A high-level overview of effector mechanisms involved in human antifungal immunity (Netea et al., 2015)

The innate immune mechanisms recruited to the frontline defence include an armament of soluble antifungal peptides, such as epithelial cells-derived β-defensins, an array of PRR on various cells, such as C-type lectin receptors (dectins 1, 2, 3), TLR, NLR and RLR receptors, mannose receptors DC-SIGN and MINCLE, as well as neutrophil-bound Complement receptor 3 and Fcγ receptors. The engagement of these receptors via the *Candida* PAMPS, such as O/N-mannans, beta-glucans or chitins, initiates signalling events, which mediate and amplify the danger signal; for instance, the activation of SYK- and RAF1-dependent pathways [utilizing the caspase activation and recruitment domain-containing 9 adaptor protein (CARD9) (Drummond et al., 2011)], activation of protein kinase Cδ (PKCD) (Elsori et al., 2011), the NF-κB pathway, the RLR melanoma differentiation-associated protein 5 (MDA5)-mediated pathway, or the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome activation pathway (Netea et al., 2015). Interestingly, NLRP3 inflammasome is only activated by *Candida* hyphae, but not yeasts, which represents one of the discriminatory mechanisms of host response to harmless *Candida* colonization and invasion (Gow et al., 2012).

The overall effects of these primary immune events are the production of anti-*Candida* microbicidal peptides, pro-inflammatory cytokines (such as IL-1β, IL-6, IL-17, IL-22, IL-23, TNF-α) and chemokines, the recruitment of neutrophils, phagocytes and dendritic cells to the site of invasion, as well as activation of T lymphocytes. The immune cells involved in the protection against systemic *Candida* invasion are mainly neutrophils (via production of ROS, fungicidal lysozyme, lactoferrin, elastase, cathepsin G or via formation of neutrophil extracellular traps), monocytes (via their proinflammatory secretory activity, bloodstream patrolling and ability to differentiate into macrophages) and macrophages themselves (via oxidative and non-oxidative killing mechanisms and their production of proinflammatory cytokines). On the other hand, mucosal surfaces are guarded by innate lymphoid cells (ILC) (i.e., IFNγ- producing ILC1 and IL-17/22 producing ILC3) and IFNγ-, IL-17-, and IL-22-producing T cells, activated by *Candida* peptides processed and presented by dendritic cells via their main histocompatibility complex class II (Netea et al., 2015).

As for the adaptive immune mechanisms, type 1 helper T cells producing IFNγ are important for the activation of macrophages and neutrophils, however the pivotal role is carried out by a subset of T helper lymphocytes called Th17. These cells are induced under the influence of TGF-β, IL-6, IL-21 and IL-23. The three latter signal via STAT3, which upregulates the expression of Th17 master regulator, *RORC*, encoding the retinoic acid-related orphan receptor γ (RORγ), which then in turn acts as a lineage-specific transcription factor inducing Th17-related products IL-17F, IL-17A, IL23R (Chi et al., 2022). These factors subsequently promote epithelial cells' and neutrophils' responses

to *Candida* on the cutaneous and mucosal surfaces (van de Veerdonk et al., 2011; Liu et al., 2011; Puel et al., 2011). The events following the sensing of *Candida* are schematically summarized in Figure 17.

Antibody-mediated immunity probably plays only a minor role in *Candida* protection, as evidenced by the absence of increased fungal susceptibility in patients with inborn agammaglobulinemia. Some utility of antibodies is, however, suggested by that fact the immunoglobulin-opsonized *Candida* is recognized by the Fcγ receptors, which enhances its phagocytosis (Gazendam et al., 2014).

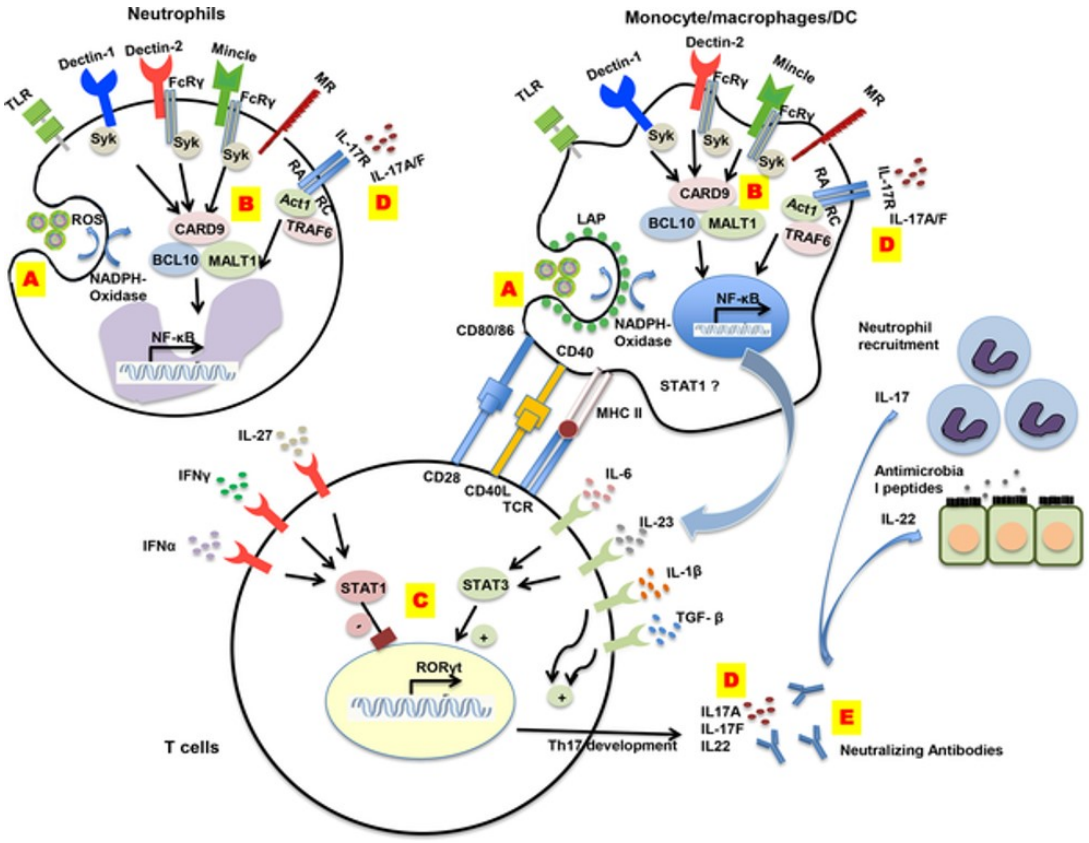


Figure 17. A cell-level overview of the key aspects of human antifungal immunity (details in the text) (Wang and van de Veerdonk, 2016)

1.4.10.3 Genetic host factors of Candida susceptibility

Infections with *Candida* have long been known to accompany many classic IEI, which display additional clinical features and/or broader infectious susceptibility beyond the propensity to *Candida.* In respect to chronic mucocutaneous candidiasis (CMC), these may be categorized as "syndromic CMC" and "CMC associated with other IEI" (as opposed to truly "isolated" CMC) and

include IEI with compromised T cell compartment, such as SCID, STAT1 gain-of-function (GOF) CMC, AD HyperIgE syndrome, ZFX341 deficiency, RORγ deficiency, CARD9 deficiency, autoimmune polyglandular syndrome type 1 (APS1 or AIRE deficiency), and neutrophil defects, such as chronic granulomatous disease and disorders of neutrophil development (Figure 18).

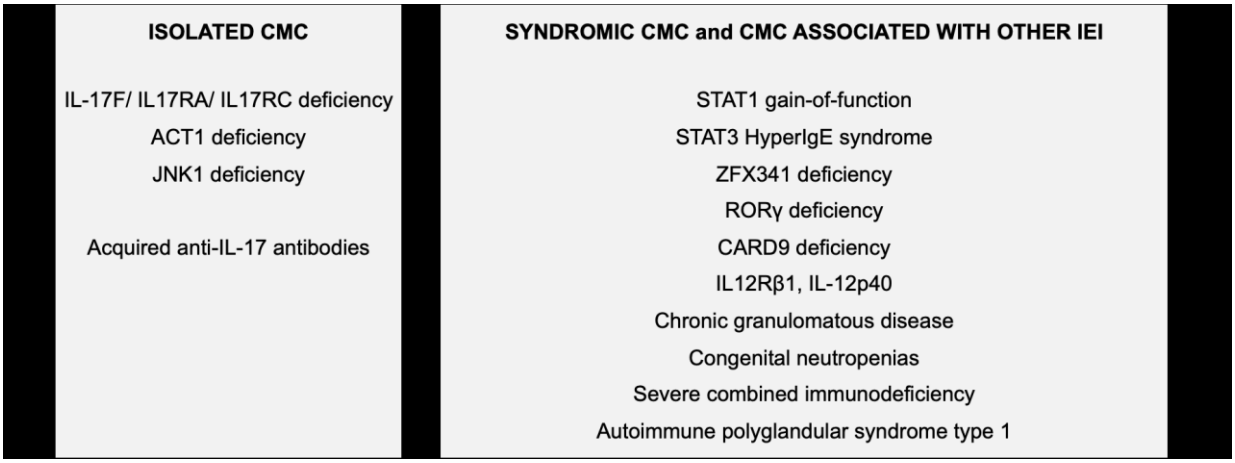


Figure 18. Aetiologies of chronic mucocutaneous candidiasis, presenting as either singularly increased susceptibility to *Candida* (column on the left), or associated with other features or IEI (column on the right)

The first two genetic aetiologies of "isolated" candidiasis were found to underlie AR IL-17 receptor A deficiency and AD IL-17F deficiency in 2011 (Puel et al., 2011b). The discoveries provided a long-expected proof of a principle that IL-17 and its signalling sequalae are essential for mucocutaneous immunity against *C. albicans*, but redundant for other antimicrobial immunity. To date, mutations in *IL17RA, IL17F, IL17RC, ACT1* and *JNK1* are known to underlie the isolated CMC (Puel, 2020). Correspondingly, many of the CMC symptoms in other IEI may be explained by the impairment of Th17 induction or activities. For instance, the failure of Th17 induction in AD HyperIgE syndrome is due to the lack of STAT3 which fails to mediate signalling downstream from IL-6, IL-21 and IL-23 receptors (Ma et al., 2008); in STAT1 GOF the functional dominance of STAT1 over STAT3 and the inhibitory effect of type I IFN-rich environment on STAT3 underlies the Th17-penia; in AR ZNF341 deficiency and RORγ deficiency low Th17 counts are due to the loss of STAT3 transcription regulator and the loss of Th17 transcription factor, respectively (Ma et al., 2008; Okada et al., 2015). The restriction of IL-17 and IL-22 activity in APS1 is due to the presence of autoantibodies against these cytokines (Puel et al., 2010). Interestingly, phenocopies of monogenic isolated CMC were reported in adult-onset CMC cases due to the high level of neutralizing autoantibodies against

cytokines IL-17A, IL-17F and IL-22 (Ku et al., 2020). Finally, a secondary "isolated" CMC is sometimes experienced by patients treated for autoimmune disorders with monoclonal antibodies targeting anti-IL-17 (Báez-Gutierrez and Rodríguez-Ramallo, 2021).

Interestingly, only a small fraction of CMC patients develops invasive candidiasis. The relative scarcity of invasive candidiasis in patients with profound T cell defects (who, on the other hand, frequently suffer from CMC) underscores the limited role of T cells in prevention of systemic *Candida* invasion, but their pivotal role in defence against surfaces-restricted infections. Conversely, invasive candidiasis is more common in neutrophil disorders, monocyte/macrophage disorders, or CARD9 deficiency, which, interestingly also predisposes to invasive dermatophytosis (Puel, 2020; Vinh, 2011).

The following text describes in further detail a rare syndromic CMC caused by hypermorphic mutations in *STAT1* (STAT1 GOF) as this disease was at the centre of this author's doctoral research. The first Czech patient with STAT1 GOF CMC was diagnosed in 2015. At the time of conception of this thesis, 12 patients with STAT1 GOF CMC have been identified, most of them are managed by the author of this thesis.

1.4.10.4 STAT1 gain-of-function chronic mucocutaneous candidiasis (STAT1 GOF CMC)

The hypermorphic mutations in *STAT1* were originally described to convey a selective susceptibility to superficial *Candida* infections with some autoimmune features (van de Veerdonk et al., 2011). To date, over 400 patients have been reported worldwide, harbouring a total of over 100 mutations. Intriguingly, STAT1 GOF was found to underlie over half of all inherited cases of CMC. Studies on larger cohorts also demonstrated that the mutations results in syndromic disease with a complex and heterogeneous set of infectious and non-infectious symptoms (Toubiana et al., 2016; Liu et al., 2011). This diversity of clinical features is not understood; however, a variant-specific gene transcription profile was reported in some mutations (Giovannozzi et al., 2021; Ovadia et al., 2018).

The individuals affected by STAT GOF mutations suffer from extensive, recurrent or refractory CMC (Figure 19). The risk of invasive candidiasis is also increased (in ~10% of cases), and the infectious spectrum is wider, i.e., patients suffer from other fungal, viral and bacterial pathogens. Moreover, up to 40% of patients develop autoimmune complications (such as thyroiditis, immune cytopenia, hepatitis, systemic lupus-like symptoms, diabetes mellitus, Addison disease), some

present with vasculopathies (large vessel aneurysms in 6% of patients), and skin/mucosal malignancies (in 6% of patients).

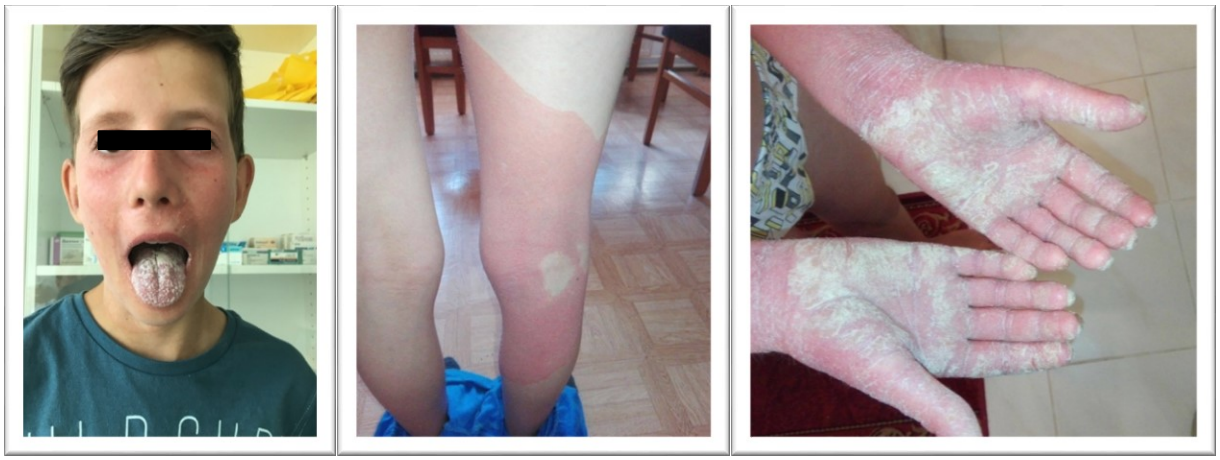


Figure 19. Clinical presentation of chronic mucocutaneous candidiasis in a patient with STAT1 gain-of-function mutation (archives of the author)

The median age of onset is approximately one year, although the diagnosis is often delayed until adulthood (Depner et al., 2016; Toubiana et al., 2016; Okada et al., 2020; Puel et al., 2011). In fact, a significant proportion of patients with milder CMC symptoms is diagnosed only when their offspring displays similar symptoms, as STAT1 GOF segregates in AD trait. On the other hand, the disease is incompletely penetrant, and minority of cases arise from *de-novo* mutations.

The pathophysiology has not been fully explained yet. While the increased fungal susceptibility is now well linked to the failure of Th17 immunity (Zheng et al., 2015), the non-infectious features of STAT1 GOF CMC are much less understood, particularly the autoimmune and vascular complications. On a molecular level, the increased STAT1 activity is proposed to be caused by one, or a combination of the following: augmented STAT1 phosphorylation, delayed STAT1 dephosphorylation, increased total STAT1 protein levels or increased stability of the STAT1 dimer (Zheng et al., 2015; Zimmerman et al., 2019). These events likely result in altered histone acetylation upon STAT1-DNA interaction. Consequently, the transcription of STAT3-inducible genes is reduced, as STAT1 and STAT3 compete for the DNA-binding sites (Zheng et al., 2015). Thus, the STAT3-mediated Th17 differentiation is hampered, and the Th17-dependent defence against *Candida* is impaired.

On the other hand, the pathophysiological background of autoimmune features in STAT1 GOF is only hypothesized. Several theories, derived mainly from similarities with other IEI with diverse autoimmune dysregulation, such as APS1 syndrome (Zimmerman et al., 2017),

immunodysregulation-polyendocrinopathy-enteropathy X-linked syndrome (IPEX syndrome) (Bennett et al., 2001)or type I interferonopathies (eg., Aicardi-Goutières syndrome, systemic lupus erythematodes) (Crow, 2011; Crow and Manel, 2015) were suggested. The presence of autoantibodies or intrinsic defects of B cells and their signalling (Romberg et al., 2017; Hiller et al., 2018), the role of regulatory cells (Treg) (Uzel et al., 2013), and the pro-autoimmune bias of the enhanced type I and II signalling via the enhanced activity of STAT1 (Okada et al., 2020) were examined, alas, none of these mechanisms have provided an overarching explanation.

The baseline immunologic work-up may yield normal results, even in cases with extensive CMC. Nevertheless, dysgammaglobulinaemia (most commonly low IgG2 and IgG4) and T or B lymphopenia (most frequently decreased memory B lymphocytes) are regular findings. These are likely secondary sequalae of the chronic inflammatory milieu, rather than primary consequences of the STAT1 mutation. In terms of life-threatening systemic *Candida* invasion, the most dangerous setting is a concurrence with immune neutropenia, which allows a swift breach of the host anti-*Candida* defence.

The diagnosis is established by genetic analysis; nowadays, NGS methods are employed for targeted single gene/multiple-gene panel/ or whole exome sequencing (Casanova et al., 2014; Ovadia et al., 2018; Xie et al., 2022). Supporting evidence is the low peripheral blood Th17 count, which may be determined with and without stimulation with mitogens (phorbol myristate acetate) or specific antigens (*Candida*, PPD, zymosan etc.), STAT1 phosphorylation assays and gene expression studies (Bloomfield et al., 2018).

The therapeutic mainstay is a long-term prophylactic administration of antifungals (Figure 20) and vigorous antimicrobial treatment of all infections. In neutropenic patients, granulocyte/granulocyte-monocyte growth factors may be used, and in the case of hypogammaglobulinemia, polyclonal immunoglobulins are administered (Toubiana et al., 2016; Depner et al., 2016).

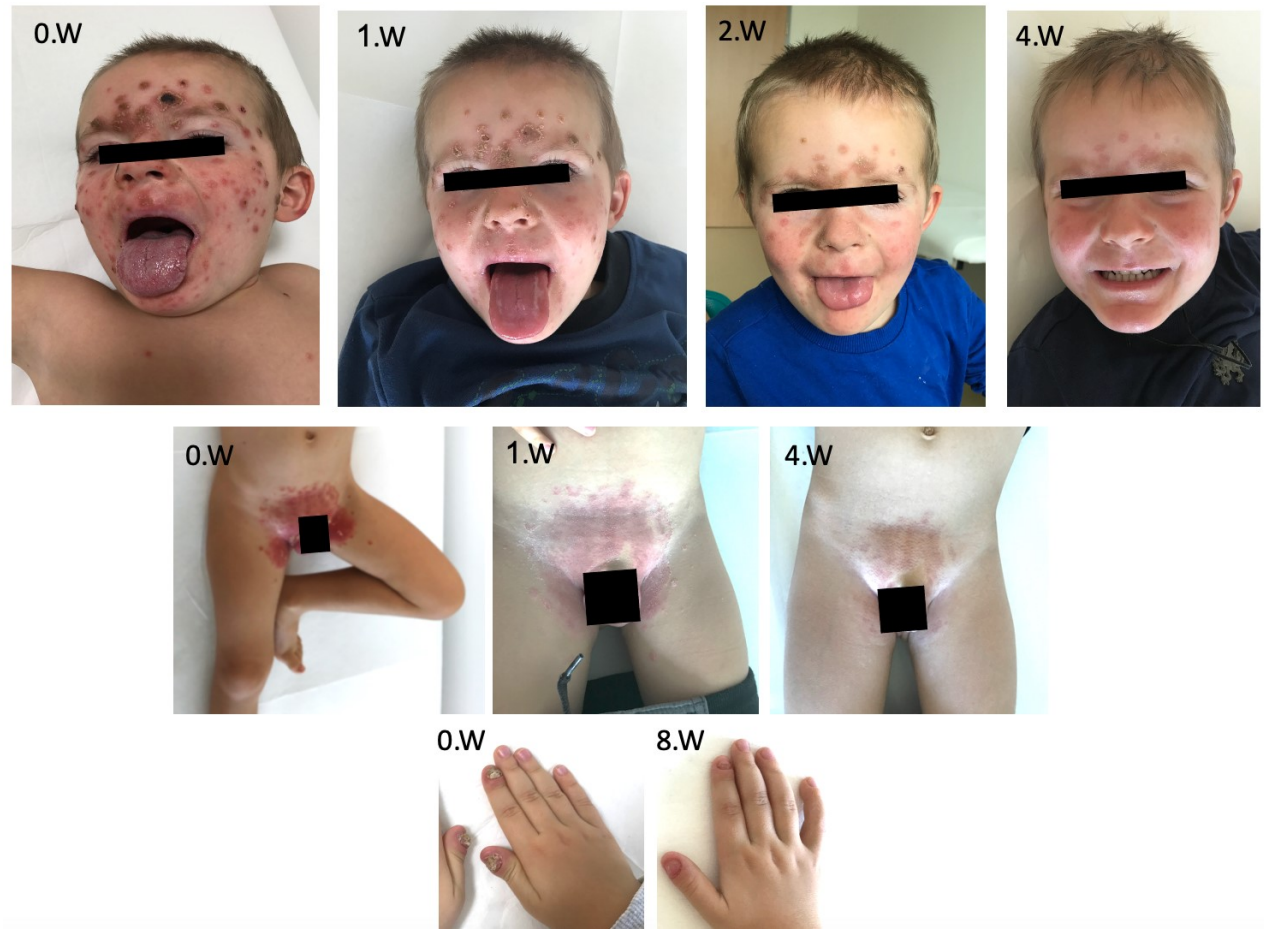


Figure 20. Effect of treatment with antifungal itraconazole on chronic symptoms of mucocutaneous candidiasis in a child with STAT1 gain-of-function mutation (archives of the author)

The latest progress suggested the possible utility of small molecules, which directly impact the immunopathologic mechanism of the disease via targeting the upstream Janus kinases (Forbes et al., 2018; Bloomfield et al., 2018; Deyà-Martínez et al., 2022). These JAK inhibitors (e.g., ruxolitinib, baricitinib) block the ability of JAK to phosphorylate STAT1, thus reducing its activity and ameliorating the symptoms of CMC and autoimmunity. Recently, sporadic data implied a curative potential of HCST (Leiding et al. ,2017; Kiykim et al., 2019), which is, however, burdened with a high risk of secondary graft failure, possibly due to abnormally increased type I and II interferon signalling. While the overall 1 year survival was only 40% (Leiding et al., 2017), the HSCT was performed as "ultimum refugium" in the majority of reported patients. It is, therefore, plausible, that a pre-emptive approach in a clinically stable conditions would yield better results.

AIMS OF THE THESIS

The key objectives of this thesis are the following:

Primary objectives

A) To describe selected clinical, immunologic, and genetic aspects of patients with **STAT1-gain-of-function chronic mucocutaneous candidiasis** and translate the findings into their clinical management

B) To describe selected clinical, immunologic, and genetic aspects of patients with **Mendelian susceptibility to mycobacterial diseases** and translate the findings into their clinical management

C) To report a proof-of-principal case that **systemic *Staphylococcus aureus* infection in individuals with disturbed IL-6 signalling** may arise due to the presence of IL-6 autoantibodies

D) To contribute to investigations concerning the novel severe **Paediatric inflammatory multisystem syndrome – temporally associated with SARS-CoV-2** (PIMS-TS; also MIS-C - Multisystemic inflammatory syndrome in children)

Secondary objectives

E) To increase awareness of IEI with selective microbial susceptibility, encourage referrals of suspect individual to immunologists and promote national cooperation

F) To connect with the international network of clinicians and researchers working in the fields of rare IEI to maximize the benefit of collective experience

METHODS

Patients

The majority of patients described in this thesis are followed by the author at the Department of Immunology, Motol University Hospital in Prague and Department of Paediatrics, Thomayer University Hospital, Prague. Some individuals are regularly followed at other departments across the country. Informed consents with inclusion in the respective research projects were obtained from the participants and/or by the participants' legal guardians in accordance with the Declaration of Helsinki.

Data on demographics, clinical manifestations, routine laboratory features and other investigations, therapeutic management, and outcomes were collected from the medical records and obtained via patient/parent interview or communications with other patients' healthcare providers. International data sharing was based on personal communications of the author of this thesis.

Laboratory methods

Laboratory, analytic and statistical methods were used according to the individual aim of each presented study and performed at the Department of Immunology, Motol University Hospital in Prague, Childhood Leukaemia Investigation Prague laboratory and the genetic laboratory in Centre for Cardiovascular Surgery and Transplantation, Masaryk University, Brno. Some genetic evaluations were performed in Laboratoire de Génétique Humaine des Maladies Infectieuses, Institut National de la Santé et de la Recherche Médicale et Université Paris Descartes, France.

The laboratory methods are described in detail in the manuscripts which substantiate this thesis. In general, these included flow cytometry methods, immunoassays and various techniques of DNA and RNA sequencing.

RESULTS

1.1 PRIMARY ENDPOINTS - PUBLICATIONS DIRECTLY SUPPORTING THE THESIS

This section introduces the following publications which directly substantiate the thesis:

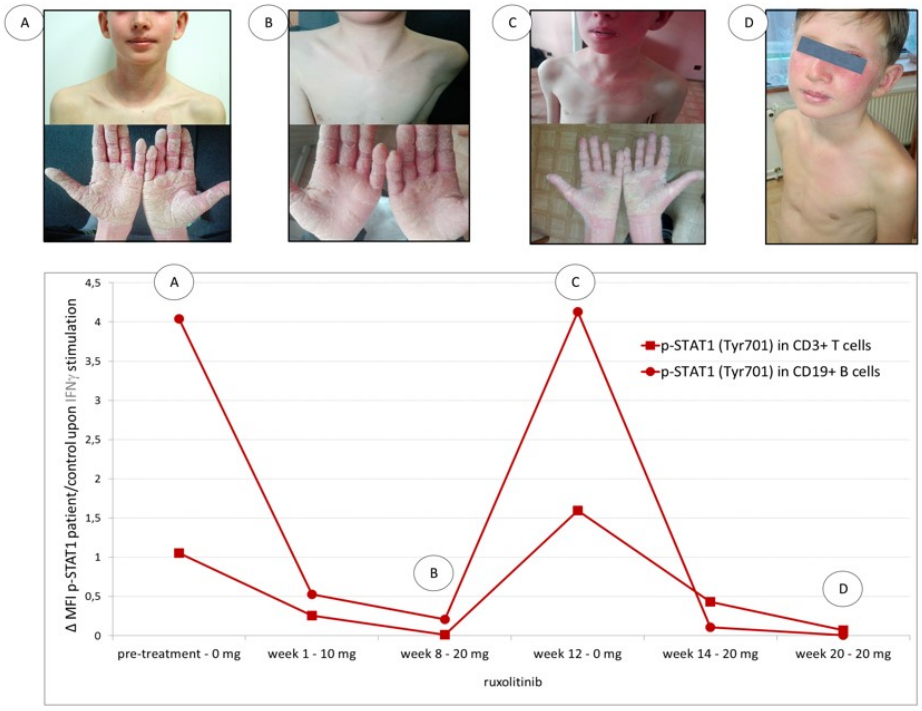
1. Utility of ruxolitinib in a child with chronic mucocutaneous candidiasis caused by a novel STAT1 gain-of-function mutation
2. Impact of JAK inhibitors in pediatric patients with STAT1 Gain of function (GOF) mutations-10 children and review of the literature
3. Immunogenicity and Safety of COVID-19 mRNA Vaccine in STAT1 GOF Patients
4. Mutual alteration of NOD2-associated Blau syndrome and IFNγR1 deficiency
5. Manifestations of cutaneous mycobacterial infections in inborn errors of IL-12, IL-23/IFNγ immunity
6. Mendelian susceptibility to mycobacterial disease: The first case of a diagnosed adult patient in the Czech Republic
7. Anti-IL6 autoantibodies in an infant with CRP-less septic shock
8. Nationwide observational study of paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the Czech Republic
9. EAACI statement and guideline on the pathogenesis, immunology, and immune-targeted management of the Multisystem inflammatory syndrome in children (MIS-C) or Pediatric inflammatory multisystem syndrome (PIMS-TS)
10. B cells, BAFF and interferons in MIS-C

1.1.1 UTILITY OF RUXOLITINIB IN A CHILD WITH CHRONIC MUCOCUTANEOUS CANDIDIASIS CAUSED BY A NOVEL STAT1 GAIN-OF-FUNCTION MUTATION

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. Utility of Ruxolitinib in a Child with Chronic Mucocutaneous Candidiasis Caused by a Novel STAT1 Gain-of-Function Mutation. *J Clin Immunol.* 2018 Jul;38(5):589-601.

In this article, the author and her colleagues reported a novel STAT1 mutation to underlie features of extensive CMC. We established the hypermorphic effect of the mutation by employing single-cell STAT phosphoflow assay, which was developed by the authors. Moreover, the paper was one of the first to described the utility of targetted therapy of STAT1 GOF with JAK inhibitor ruxolitinib in pediatric settings, and the first to monitor the clinical effect of the compound in parallel to the cellular responses to JAK inhibitor. The optimized phosphoflow protocol was then used for treatment monitoring prior to this child HSCT, and for dose adjustements in three other STAT1 GOF patients who received JAK inhibitor.

Achieved key objectives = A, E



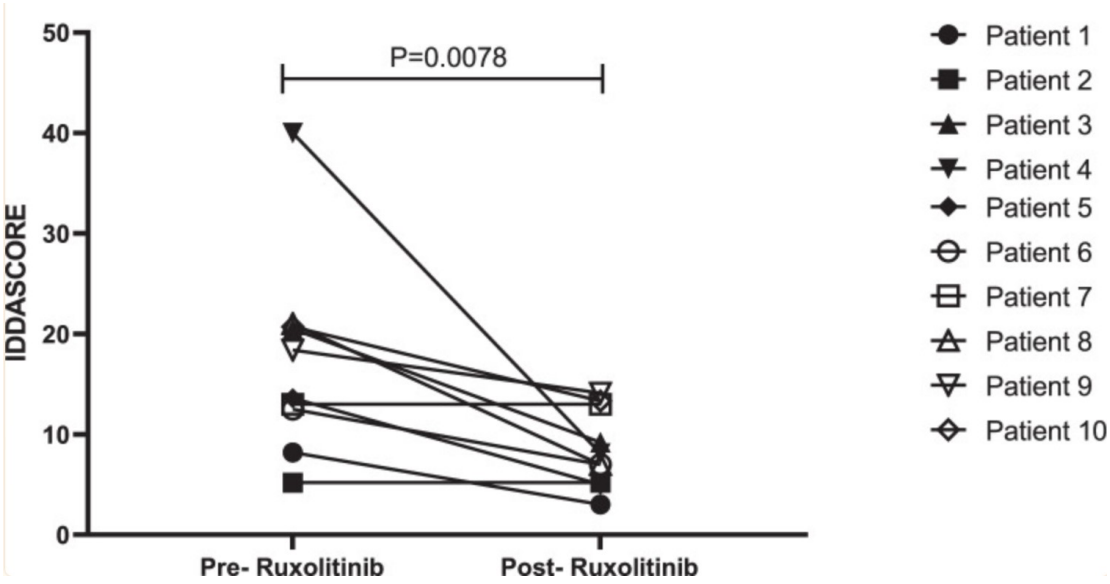
Representative figure: Clinical progress paralleled to IFNγ-induced p-STAT1 (Tyr701) activation during ruxolitinib treatment of STAT1$^{c.617T>C}$ patient

1.1.2 IMPACT OF JAK INHIBITORS IN 10 PEDIATRIC PATIENTS WITH STAT1 GAIN-OF-FUNCTION MUTATIONS (STAT1 GOF) AND REVIEW OF THE LITERATURE

Deyà-Martínez A, Rivière JG, Roxo-Junior P, Ramakers J, **Bloomfield M**, Guisado Hernandez P, Blanco Lobo P, Abu Jamra SR, Esteve-Sole A, Kanderova V, García-García A, Lopez-Corbeto M, Martinez Pomar N, Martín-Nalda A, Alsina L, Neth O, Olbrich P. Impact of JAK Inhibitors in Pediatric Patients with STAT1 Gain of Function (GOF) Mutations-10 Children and Review of the Literature. *J Clin Immunol.* 2022 Jul;42(5):1071-1082.

This international collaborative study arose from the applicant's communications with colleagues from Spain and Brazil and concerned the experience with efficacy and safety of precision treatment of paediatric STAT1 GOF patients with JAK inhibitors. Prior to this publication, such reports had been scarce, limited to individual reports. Based on our collective experience, this group of authors formed recommendations regarding dosing, monitoring, and follow-up care and envisaged paths for future clinical research, such as drug level monitoring or the identification of treatment-response biomarkers. The group has since become involved in the European Society for Immunodeficiency (ESID) and European Society for Blood and Marrow Transplantation (EBMT) multicentric retrospective study on JAK inhibitors treatment in patients with inborn errors of the JAK/STAT pathways.

Achieved key objectives = A, F



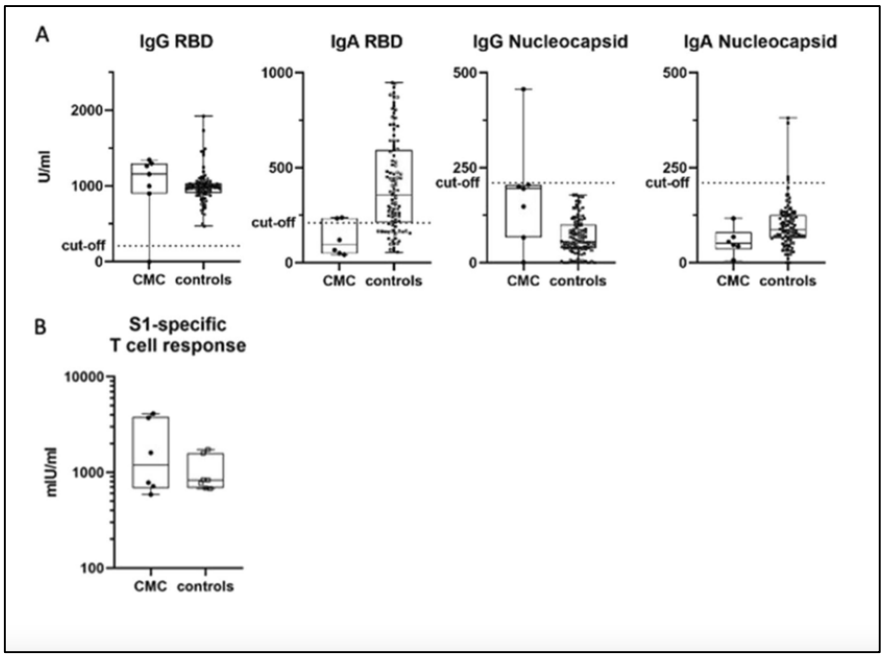
Representative figure: Effect of JAK inhibitor ruxolitinib on Immune deficiency and dysregulation activity (IDDA) score in STAT1 GOF pediatric patients

1.1.3 IMMUNOGENICITY AND SAFETY OF COVID-19 mRNA VACCINE IN STAT1 GOF PATIENTS

Bloomfield M, Parackova Z, Hanzlikova J, Lastovicka J, Sediva A. Immunogenicity and Safety of COVID-19 mRNA Vaccine in STAT1 GOF Patients. *J Clin Immunol.* 2022 Feb;42(2):266-269.

The emergence of COVID-19 brough on serious concerns about its course in patients with IEI. On the other hand, the availability of mRNA vaccines against SARS-CoV-2 raised questions about their safety and efficiency in individuals with IEI. It had been altogether unknown, if the STAT1 GOF IFN-augmented environment would be protective or detrimental in case of SARS-CoV-2 infection and vaccination, as, hypothetically, both may increase the risk of the catastrophic cytokine-driven hyperinflammation seen in delayed stages of COVID-19 in some patients. At the time when only three records of COVID-19 infection and only two records of mRNA vaccinations in STAT1 GOF existed worldwide, we reported seven STAT1 GOF patients with an uneventful course of COVID-19 vaccination (including data on antibody and T-cell mediated responses), and/or SARS-CoV-2 infection, including two patients receiving JAK inhibitor. Additionally, two of the patients described in this publication harbour previously unreported mutations, which expanded the known STAT1 GOF- associated variant pool.

<u>Achieved key objectives = A, E</u>



Representative figure: Immune responses to SARS-CoV-2 mRNA vaccine in STAT1 GOF patients after the second vaccine dose

1.1.4 MUTUAL ALTERATION OF NOD2-ASSOCIATED BLAU SYNDROME AND IFNYR1 DEFICIENCY

Parackova, Z*, **Bloomfield***, Vrabcova P, Zentsova I, Klocperk A, Milota T, Svaton M, Casanova JL, Bustamante J, Fronkova E, Sediva A. 2019. Mutual alteration of NOD2-associated Blau syndrome and IFNγR1 deficiency. *J. Clin. Immunol.* 2020; 40:165–178.

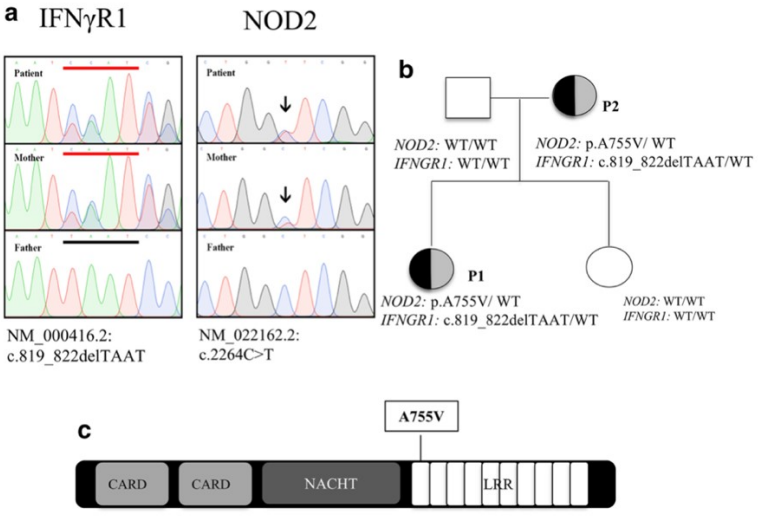
*Authors contributed equally

In this work, we followed an extraordinary experiment of nature – a unique kindred harbouring heterozygous mutations in two innate immune mechanisms of antimicrobial defences - IFNGR1 (associated MSMD) and NOD2 (associated with Blau syndrome). Together, they resulted in a combined phenotype of milder MSMD and atypical Blau syndrome. This was intriguing, because NOD2 Blau syndrome, an auto-inflammatory granulomatous disease of unknown pathophysiology, was hypothesized to involve abnormal response to IFNγ. These two pathways have, however, not been previously mechanistically linked.

Utilizing an array of NOD2 and IFNγ pathways-probing molecular methods, we demonstrated a functional crosstalk, which suggested that IFNγ is an important driver in the NOD2 hyperreactivity in Blau syndrome, independently of IFNγR/STAT1-mediated signalling.

Our two years long effort eventually enabled intelligence-based selection of optimal therapy for the patient. Moreover, the hereby described observations contributed to the notion of therapeutic targeting of IFNγ signalling in Blau syndrome BS.

Achieved key objectives = B, F



Representative figure: DNA sequencing chromatograms, the pedigree and segregation of the *NOD2* and *IFNGR* mutations, and the NOD2 protein structure highlighting the aminoacid

substitution in the proband

We were honoured that this work was co-authored by Prof. J.L.Casanova and received personal appreciation from doctor Edward B. Blau:

Dear colleagues,
I am the Edward B. Blau …and I must tell you your article … is one of the most fascinating that I have read in many a year.
The clinical presentation, diagnosis and treatment are first rate. The laboratory work was also of the highest order.
I have always been interested in what stimulates the (deficient) NOD2 system … to go onto unchecked granuloma formation.
I have thought it might be … benign Mycobacterium. Please extend my congratulations on a splendid piece of work to your colleagues.

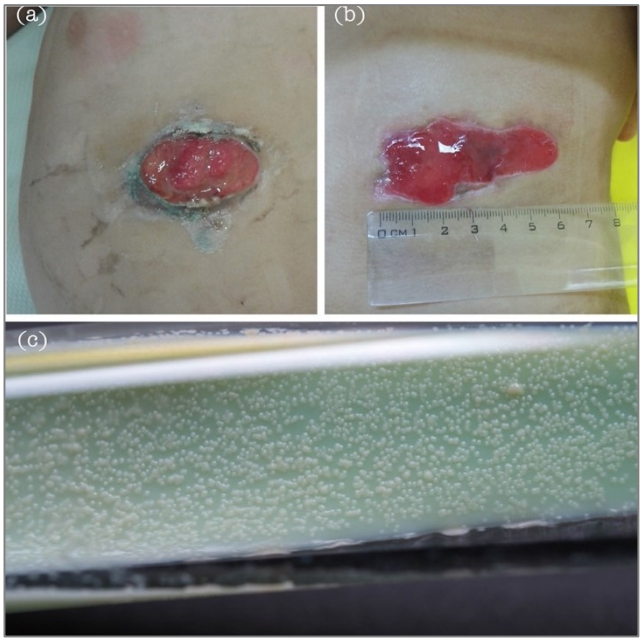
Very sincerely yours,
Edward B. Blau, M. D.
Marshfield, WI, USA

1.1.5 MANIFESTATIONS OF CUTANEOUS MYCOBACTERIAL INFECTIONS IN INBORN ERRORS OF IL-12, IL-23/IFNγ IMMUNITY

Dolezalova K, Strachan T, Matej R, Ricna D, **Bloomfield M.** Manifestations of cutaneous mycobacterial infections in inborn errors of IL-12, IL-23/IFNγ immunity. *European Journal of Dermatology*. 2022; 7;32(4):1-10.

In this manuscript, we aimed to portrait patients with MSMD from the dermatologic perspective, as individuals with disturbed IL-12, IL-23/IFNγ circuit often present with cutaneous infections with non-tuberculous mycobacteria. The mycobacteriosis in MSMD may, however, adapt an atypical or severe course, lacking the classic granulomatous nature. Collaborating with Czech and Slovakian paediatric TB specialists, geneticists and the pathologist, two main objectives of this article were set: to increase awareness of MSMD to facilitate timely referral of the suspect cases, and to highlight the characteristics of NTM infections and the pitfalls of their diagnosis in both the immunocompetent and MSMD patients. Specifically, infections with M. marinum and BCG in AD partial STAT1 deficiencies, infections with M. avium-intracellulare, M. abscessus-immunogenum and BCG in AD partial IFNγR1 deficiency, and infections with M. abscessus-intracelulare and BCG in fatal AR complete IFNγR1 deficiency were depicted. Moreover, one of the presented family harboured a previously unreported mutation in STAT1 gene, thus enriching the known disease-associated genotypes. Also, one of the children was successfully treated with IFNγ.

Achieved key objectives = B, E, F



Representative figure: *Mycobacterium avium-intracellulare* scrofuloderma of the thorax due to autosomal recessive complete IFNγR1 deficiency

1.1.6 MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES: THE FIRST CASE OF A DIAGNOSED ADULT PATIENT IN THE CZECH REPUBLIC

Prucha M, Grombirikova H, Zdrahal P, **Bloomfield M,** Parackova Z, Freiberger T. Mendelian Susceptibility to Mycobacterial Disease: The First Case of a Diagnosed Adult Patient in the Czech Republic. *Case Reports Immunol.* 2020 Dec;8836685.

This case study represents an example of a successful national collaboration. It describes a 42-year-old woman, who suffered from severe obscure mycobacterial infections most of her life. Only in her adulthood, she was referred to a genetic evaluation and found to harbour IFNGR1 mutation by the team of geneticists, who specialize in IEI. The loss-of-function consequence of the variant was validated by the author of this thesis and her colleagues. Based on previous experience and literary accounts, treatment with recombinant IFNy was recommended, which improved the clinical condition of the patient and prevented further mycobacterial infections. Effectively, this cooperation has, to the patient benefit, brought together the expertise of four different clinics and departments. The paper adds to the expanding pool of patients who are diagnosed with inborn immunodeficiency in later adulthood and reports the first adult Czech patient diagnosed with penetrant MSMD.

Achieved key objectives = B, E

Year	Localization	Pathogen
1981	Inguinal and cervical lymph nodes	*M. kansasii*
1992	Lungs	Wrongly diagnosed as sarcoidosis
1993	Lymph nodes, Th-7, 8, 9, 11, 12, L1, 2, 5 left femur, maxilla, mandibula	*M. kansasii*
1995	Centre in the distal part of the left femur	*M. avium intracellulare* *M. gordonae*
1997	Granuloma in the right face Left patella	*M. lentiflavum*
1998	Distal part of the femur on the right Granuloma in the right face	*M. lentiflavum* *M. kansasii*
2001	Granuloma of the nasal septum	*M. avium intracellulare*
2002	Left knee	*M. flavescens*
2004	Sputum	*M. lentiflavum*
2007	Granuloma/nasal septum	*M. lentiflavum*
2009	Granuloma/nasal septum	*M. lentiflavum*
2013	Granuloma/nasal septum	*M. avium*
2014	TH 8, 11, L1, L5	*M. avium*
2016	Granuloma of the nasal septum	*M. lentiflavum*
2017	Colliquating granuloma in the nasal septum	*M. avium intracellulare*
2018	Granuloma of the nasal septum Colliquating granuloma in the right face	*M. avium* Start therapy rhIFN-γ
2020	Surgery of the nasal septum Granuloma of the right face healed	Microbiological investigation is negative

Representative figure: The sequence of infections with non-tuberculous mycobacteria in patient with autosomal dominant partial IFNyR1 deficiency

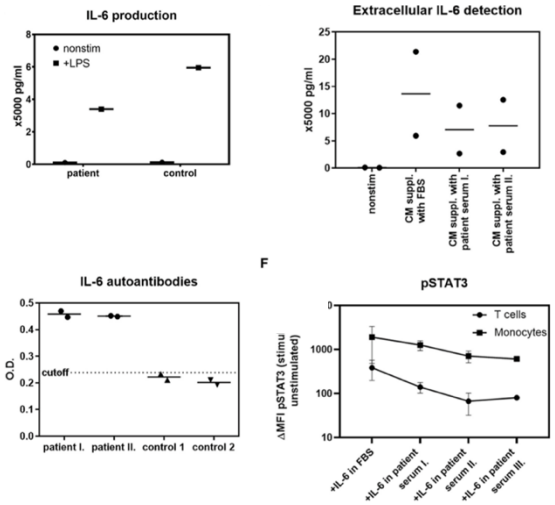
1.1.7 ANTI-IL6 AUTOANTIBODIES IN AN INFANT WITH CRP-LESS SEPTIC SHOCK

Bloomfield M*, Parackova Z*, Cabelova T, Pospisilova I, Kabicek P, Houstkova H, Sediva A. Anti-IL6 Autoantibodies in an Infant With CRP-Less Septic Shock. *Front. Immunol*. 2019;10: 1–6.
*Authors contributed equally

Certain IEI are associated with skewed inflammatory acute phase response, as well as increased susceptibility to Staphylococcus aureus (e.g., STAT3 HyperIgE syndrome, IL6R deficiency, or gp130 mutation). Examining a child with staphylococcal sepsis, who failed to mount an adequate C-reactive protein (CRP) and IL-6 response, we tested the functional integrity of IL-6/gp130/IL6R/STAT3 pathway and established that the patient's cells were able to produce and secrete normal amounts of IL-6 and displayed normal STAT3 recruitment upon IL-6 stimulation. Surprisingly, the failed CRP induction was explained by the presence of autoantibodies against IL-6 in the patient's serum. Prior to this publication, only three patients with anti-IL6 autoantibodies had been reported to suffer localized bacterial infections. As such, this work provided a proof-of principle, that systemic Staphylococcal infection, too, may arise due to disturbed IL-6 signalling on the account of naturally occurring anti-IL6 autoantibodies. Since the monogenic defects of IL-6 signalling (other than STAT3 loss-of-function) have only been reported in a handful of patients, our findings indirectly affirmed the crucial role of IL6 signalling in the anti-staphylococcal immunity. Importantly, they also translated to a larger-scale clinical issue, i.e., the need for caution in patients receiving compounds interfering with IL-6 signalling, such as those currently used for several rheumatologic, immune dysregulation diseases and cancer.

This work was received the best poster award in the 14th Paediatric congress in Olomouc in 2019.

<u>Achieved key objectives = C, E</u>



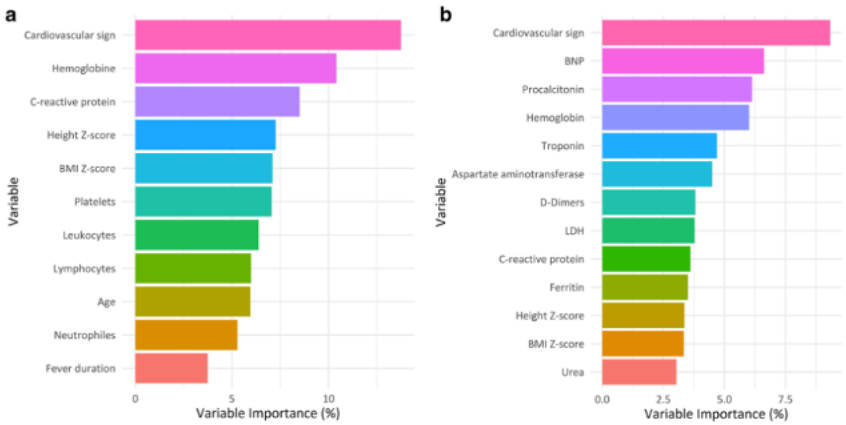
Representative figure: IL-6/IL6R/STAT3 investigations in a patient with Staphylococcal sepsis and failed induction of C-reactive protein

1.1.8 NATIONWIDE OBSERVATIONAL STUDY OF PAEDIATRIC INFLAMMATORY MULTISYSTEM SYNDROME TEMPORALLY ASSOCIATED WITH SARS-COV-2 (PIMS-TS) IN THE CZECH REPUBLIC

David J, Stara V, Hradsky O, Tuckova J, Slaba K, Jabandziev P, Sasek L, Huml M, Zidkova I, Pavlicek J, Palatova A, Klaskova E, Banszka K, Terifajova E, Vyhnanek R, **Bloomfield M,** Fingerhutova S, Dolezalova P, Prochazkova L, Chramostova G, Fencl F, Lebl J. Nationwide observational study of paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the Czech Republic. *Eur J Pediatr.* 2022 Aug; 20:1–10.

PIMS-TS (MIS-C) is a novel life-threatening disease which emerged during the COVID-19 pandemic. Its pathophysiology is unknown, yet, indisputably, the immune system dysregulation and genetic factors play the pivotal roles. As such, PIMS-TS represents another disease with predisposition to severe course of infection by a single pathogen, as all other infections seem to take on an uneventful course in these children. This retrospective nationwide observational study collected epidemiologic, clinical and laboratory data of 207 Czech children with PIMS-TS from nine university hospitals and eight regional hospitals, representing the largest cohort reported at the time of publishing. We established that the incidence of PIMS-TS out of all SARS-CoV-2-positivelly tested children was 0.9:1,000. The delay between PIMS-TS cases accumulation from the peak of the COVID-19 wave was 3 weeks. Beyond the epidemiological observations, several predictors of life-threatening myocardial dysfunction were identified. These included chiefly the clinical signs of cardiovascular involvement at the initial phases of the disease, decreased concentration of haemoglobin, thrombocytopenia, elevated concentration of CRP, procalcitonin B-type natriuretic peptide and troponin. Upon follow-up, majority of patients fully recovered and had normal cardiac function.

Achieved key objectives = D, E



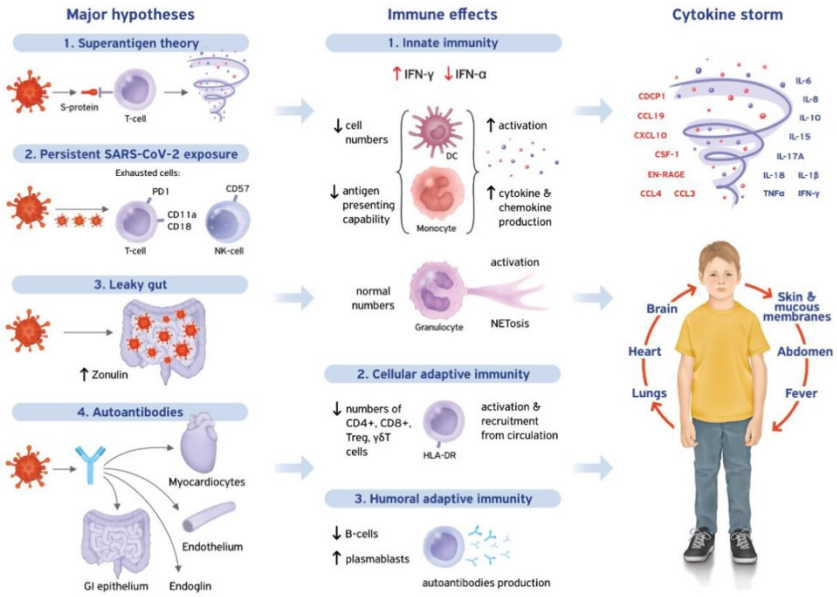
Representative figure: Predictors of myocardial dysfunction in PIMS-TS stratified by the level of healthcare provider for the use of a) general practitioners b) hospital care providers

1.1.9 EAACI STATEMENT AND GUIDELINE ON THE PATHOGENESIS, IMMUNOLOGY, AND IMMUNE-TARGETED MANAGEMENT OF THE MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C) OR PEDIATRIC INFLAMMATORY MULTISYSTEM SYNDROME (PIMS)

Feleszko W, Okarska-Napierała M, Pauline Buddingh E, **Bloomfield M,** Sediva A, Bautista-Rodriguez C, Brough HA, Eigenmann PA, Eiwegger T, Eljaszewicz A, Eyerich S, Gomez-Casado C, Fraisse A, Janda J, Jiméeneéz-Saiz R, Kallinich T, Krohn IK, Mortz CG, Riggioni C, Sastre J, Sokolowska M, Strzelczyk Z, Untersmayr E, Tramper-Stranders G. EAACI statement and guideline on the pathogenesis, immunology, and immune-targeted management of the Multisystem inflammatory syndrome in children (MIS-C) or Pediatric inflammatory multisystem syndrome (PIMS-TS). Under review in *Allergy,* 2022.

This multinational European collaborative endeavour reflected the need to address the multiple existing case definitions of MIS-C associated with SARS-CoV-2 infections and the lack of unified management guidelines. Members of European Academy of Allergology and Clinical Immunology formulated a joint statement regarding the immune aspects of MIS-C, as well as clinically practical management algorithms. Four main hypotheses of immune pathologic mechanisms were defined, involving both innate and adaptive components, i.e., the superantigen-driven hyperinflammation, persistent SARS-CoV-2 exposure, leaky gut theory allowing continuous exposure to the virus, and the presence of autoantibodies. The applicant directly contributed to this work, particularly to the section on Immunology of MIS-C, receiving the opportunity to familiarize herself with the proceedings of a Delphi-based consensus protocol.

Achieved key objectives = D, F



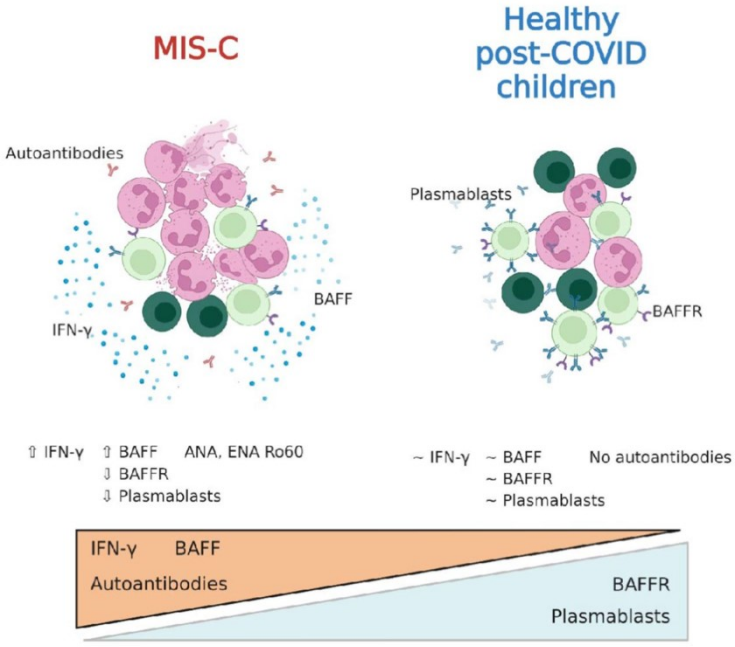
Representative figure: Overview of the hypothesized mechanisms of MIS-C (PIMS-TS) immunopathology

1.1.10 B CELLS, BAFF AND INTERFERONS IN MIS-C

Klocperk A*, **Bloomfield M*,** Parackova Z, Aillot L, Fremuth L, Sasek L, David J, Fencl F, Skotniova A, Rejlova K, Magner M, Hrusak O, Sediva A. B cells, BAFF and interferons in MIS-C. *MedRxiv preprint*, version posted May 21, 2022.
*Authors contributed equally

This work was a hypothesis-driven exploration of the involvement of B cells in the pathogenesis of MIS-C associated with SARS-CoV-2 infections in children. Parallels with clinical and immune phenotype of a classic autoimmune disorder, systemic lupus erythematosus, such as the strong interferon-based proinflammatory bias and the presence of autoantibodies suggested a disorder of B cell maturation or survival. We found elevated serum levels of B-cell activating factor (BAFF) and a counter-regulative depression of its receptor (BAFFR) on MIS-C B cells, as well as decreased proportion of mature B cells, called plasmablasts. These findings implied that a polyclonal B cell activation may be an important driver of the self-reactive phenomena accompanying MIS-C. The project connected paediatric and immunology departments of three Czech university hospitals and colleagues from Academy of Sciences, Czech Republic.

Achieved key objectives = D, E



Ilustrative figure: The involvement of humoral immunity in PIMS-TS (MIS-C) with autoreactive B cells driven towards autoantibody production by elevated BAFF

1.2 SECONDARY ENDPOINTS

During the doctoral programme, the applicant also co-authored several peer-reviewed papers published in international journals with impact factors, which explored clinical and immunopathological features of other rare IEI predisposing to infections yet displaying a broader infectious phenotype.

1. **Bloomfield M**, Klocperk A, Zachova R, Milota T, Kanderova V, Sediva A. Natural Course of Activated Phosphoinositide 3-Kinase Delta Syndrome in Childhood and Adolescence. *Front Pediatr.* 2021 Jul 19;9:697706.

2. Fejtkova M, Sukova M, Hlozkova K, Skvarova Kramarzova K, Rackova M, Jakubec D, Bakardjieva M, **Bloomfield M**, Klocperk A, Parackova Z, Sediva A, Aluri J, Novakova M, Kalina T, Fronkova E, Hrusak O, Malcova H, Sedlacek P, Liba Z, Kudr M, Stary J, Cooper MA, Svaton M, Kanderova V. TLR8/TLR7 dysregulation due to a novel TLR8 mutation causes severe autoimmune hemolytic anemia and autoinflammation in identical twins. *Am J Hematol.* 2022 Mar 1;97(3):338-351.

4. Kanderova V, Grombirikova H, Zentsova I, Reblova K, Klocperk A, Fejtkova M, **Bloomfield M**, Ravcukova B, Kalina T, Freiberger T, Sediva A. Lymphoproliferation, immunodeficiency and early-onset inflammatory bowel disease associated with a novel mutation in Caspase 8. *Haematologica.* 2019 Jan;104(1): e32-e34.

5. Smetanova J, Milota T, Rataj M, **Bloomfield M**, Sediva A, Klocperk A. Accelerated Maturation, Exhaustion, and Senescence of T cells in 22q11.2 Deletion Syndrome. *J Clin Immunol.* 2022 Feb;42(2):274-285.

6. Mensa-Vilaró A, Bravo García-Morato M, de la Calle-Martin O, Franco-Jarava C, Martínez-Saavedra MT, González-Granado LI, González-Roca E, Fuster JL, Alsina L, Mutchinick OM, Balderrama-Rodríguez A, Ramos E, Modesto C, Mesa-Del-Castillo P, Ortego-Centeno N, Clemente D, Souto A, Palmou N, Remesal A, Leslie KS, Gómez de la Fuente E, Yadira Bravo Gallego L, Campistol JM, Dhouib NG, Bejaoui M, Dutra LA, Terreri MT, Mosquera C, González T, Cañellas J, García-Ruiz de Morales JM, Wouters CH, Bosque MT, Cham WT, Jiménez-Treviño S, de Inocencio J, **Bloomfield M**, Pérez de Diego R, Martínez-Pomar N, Rodríguez-Pena R, González-Santesteban C, Soler-Palacín P, Casals F, Yagüe J, Allende LM, Rodríguez-Gallego JC, Colobran R, Martínez-Martínez L, López-Granados E, Aróstegui JI. Unexpected relevant role of gene mosaicism in patients with primary immunodeficiency diseases. *J Allergy Clin Immunol.* 2019 Jan;143(1):359-368.

7. Klocperk A, Paračková Z, **Bloomfield M**, Rataj M, Pokorný J, Unger S, Warnatz K, Šedivá A. Follicular Helper T Cells in DiGeorge Syndrome. *Front Immunol.* 2018 Jul 23;9:1730.

8. Kralickova P, Milota T, Litzman J, Malkusova I, Jilek D, Petanova J, Vydlakova J, Zimulova A, Fronkova E, Svaton M, Kanderova V, **Bloomfield M**, Parackova Z, Klocperk A, Haviger J, Kalina T, Sediva A. CVID-Associated Tumors: Czech Nationwide Study Focused on Epidemiology, Immunology, and Genetic Background in a Cohort of Patients With CVID. *Front Immunol.* 2019 Jan 22;9:3135.

9. Chovancova Z, Kralickova P, Pejchalova A, **Bloomfield M**, Nechvatalova J, Vlkova M, Litzman J. Selective IgM Deficiency: Clinical and Laboratory Features of 17 Patients and a Review of the Literature. *J Clin Immunol.* 2017 Aug;37(6):559-574.

1.3 COVID-19 INTERMEZZO

During the outbreak of SARS-CoV-2 pandemic in 2019/2020, the applicant temporarily refocused on research of COVID-19-associated immune aspects, utilizing the teams' experience with single-gene innate immune pathology, and co-authored several peer-reviewed publications in journals listed below.

This subject was of particular interest to the applicant, as a proportion of severe/fatal COVID-19 infections was shown to be the result of inborn error of type I interferon and TRL7 signalling. The applicant therefore participated in a global effort led by Dr. Casanova and Dr. Shen-Ying to ellucidate the genetic background behind severe COVID-19 and MIS-C. The resulting publications are co-authored by the applicant as part of a large consortium of clinicians involved in *Covid Human Genetic Effort* project. The applicant contributed by material and data collection. These works and listed in *italic* below, for completeness sake.

The applicant further contributed to MIS-C research by entering the HyperPed COVID Registry project, a retrospective multinational observational study, and a global research consortium on MIS-C led by colleagues from Imperial Collage, London and Rockefeller University, New York. The results from these collaborations are pending.

1. **Bloomfield M**, Pospisilova I, Cabelova T, Sediva A, Ibrahimova M, Borecka K, Magner M. Searching for COVID-19 Antibodies in Czech Children-A Needle in the Haystack. *Front Pediatr.* 2020 Nov 12;8:597736.

2. Parackova Z, Zentsova I, **Bloomfield M**, Vrabcova P, Smetanova J, Klocperk A, Mesežnikov G, Casas Mendez LF, Vymazal T, Sediva A. Disharmonic Inflammatory Signatures in COVID-19: Augmented Neutrophils' but Impaired Monocytes' and Dendritic Cells' Responsiveness. *Cells.* 2020 Sep 29;9(10):2206.

3. Parackova Z, **Bloomfield M**, Klocperk A, Sediva A. Neutrophils mediate Th17 promotion in COVID-19 patients. *J Leukoc Biol.* 2021 Jan;109(1):73-76.

4. Milota T, Sobotkova M, Smetanova J, **Bloomfield M**, Vydlakova J, Chovancova Z, Litzman J, Hakl R, Novak J, Malkusova I, Hanzlikova J, Jilek D, Hutyrova B, Novak V, Krcmova I, Sediva A, Kralickova P. Risk Factors for Severe COVID-19 and Hospital Admission in Patients with Inborn Errors of Immunity - Results from a Multicenter Nationwide Study. *Front Immunol.* 2022 Feb 28;13:835770.

5. Klocperk A, **Bloomfield M**, Parackova Z, Zentsova I, Vrabcova P, Balko J, Meseznikov G, Casas Mendez LF, Grandcourtova A, Sipek J, Tulach M, Zamecnik J, Vymazal T, Sediva A.

Complex Immunometabolic Profiling Reveals the Activation of Cellular Immunity and Biliary Lesions in Patients with Severe COVID-19*. J Clin Med.* 2020 Sep 17;9(9):3000.

6. *Zhang et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4570.*
7. *Bastard et al. Autoantibodies neutralizing type I IFNs are present in ~ 4% of uninfected individuals over 70 years and account for ~ 20% of COVID-19 deaths. Science Immunology. 2021 Aug 19;6(62):eabl4340.*
8. *Bastard et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4585.*
9. *Asano et al. X-linked recessive TLR7 deficiency in 1% of men under 60 years with life-threatening COVID-19. Science Immunology. 2021 Aug 19;6(62):eabl4348.*
10. *Sancho-Shimizu et al. SARS-CoV-2–related MIS-C: A key to the viral and genetic causes of Kawasaki disease? J Exp Med. 2021 Jun 7;218(6):e20210446.*

DISCUSSION

The modern studies of pathogenesis of ultrarare IEI teach us that the intricate complexity of our immune system clockwork is much wider than originally appreciated. The exploration of human genetic factors in the host-pathogen interactions and their defects offers unique opportunities to gain new insights into immune system composition and orchestration of its operational processes. This particularly resonates when clinically severe symptoms are not accompanied by any apparent abnormalities in routinely performed immunologic examinations, such as in the diseases addressed by the hereby presented research. Such patients, often initially reported in single-case studies, not seldomly unveil novel immune mechanisms and functionalities or confirm pre-existing hypotheses, which then drives multidisciplinary research. Given the interconnectivity of immune processes with each other, with other human biological systems and with the microbial biosphere, the translational potential of any new discovery is incontestable. However, it is a long route from the discovery of a novel gene, protein or mechanistic interaction to the verification of its causality and to the development and deployment of a new therapeutic strategy. In between, a long-term patient follow-up, disease course and treatment response monitoring provide additional data. If backed by multicentre collaborations, even the "rare" eventually becomes strong enough to build a thorough apprehension. In this thesis, the applicant hoped to participate in these efforts by some such insights.

Each work's findings are discussed briefly in the Results section above and, in detail, in the attached manuscripts which substantiate this thesis.

CONCLUSIONS

This work contributed to the understanding of three rare IEI and one disease with presumed immunogenetic background, all with selective microbial susceptibility.

With the continuing emergence of new infectious diseases, as witnessed globally during the COVID-19 pandemic in 2019, and the alarmingly increasing resistance of pathogens to currently available antimicrobial compounds, the need for continuing investigations of the immune antimicrobial mechanisms is sorely evident and must receive major attention. In the future, the author hopes to continue to contribute to research activities concerning monogenic susceptibility to individual microbes and other rare diseases with antimicrobial immune failures.

SOUHRN (SUMMARY IN CZECH)

Výsledky předkládané v této dizertační práci přispívají k porozumění imunopatologie několika vrozených poruch imunity se zvýšenou náchylností ke konkrétnímu infekčnímu patogenu:

A) u **STAT1 gain-of-function chronické mukokutánní kandidózy** byly popsány nové mutace, imunoprofilace, klinické a buněčné odpovědi na novou efektivní terapii JAK inhibitorem, využití nově vyvinutého fosfoflow protokolu a imunogenicita a bezpečnost mRNA vakcíny proti COVID-19
B) u **Vrozené vnímavosti k mykobakteriálním onemocněním** byly popsány nové mutace, jejich klinické a imunopatologické aspekty a terapeutické využití IFNy
C) u sepse způsobené **Staphylococcus aureus** byly jako příčina selhání obranyschopnosti se systémovým projevem identifikovány **autoprotilátky proti IL-6**, jako první taková reference vůbec
D) u nového onemocnění **PIMS-TS** (**Paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2)** byla publikována epidemiologická data, prediktory závažnosti průběhu onemocnění, popsány imunopatologické mechanismy týkající se autoreaktivních charakteristik B lymfocytů a navrženy doporučené postupy pro klinickou praxi

Autorka se na těchto studiích účastnila tvorbou hypotéz, designem studijních protokolů, sběrem a analýzou dat a klinickou péčí o pacienty. Dále ustanovila pacientské kohorty, navázala národní a mezinárodní spolupráci a po dobu studia aktivně prezentovala na tuzemských i zahraničních konferencích.

Výsledky se v řadě případů podařilo přenést přímo do klinické praxe; umožnily mimo jiné stratifikaci pacientů podle rizik, tvorbu individualizovaných léčebných plánů „na míru“ pacientovi, vč. konkrétních preventivních opatření, cílené terapeutické a profylaktické medikace, léčebných zákroků a možnosti poskytnutí genetického poradenství.

SUMMARY

The data presented in this dissertation thesis expanded the understanding of immunopathology underling several IEI with increased susceptibility to single infectious pathogens:

A) in **STAT1 gain-of-function chronic mucocutaneous candidiasis** novel mutations, corresponding immune profiles, clinical and cellular responses to novel, efficient therapy with JAK inhibitors, the utility of a newly developed phosphoflow protocol, and immunogenicity and safety of COVID-19 vaccination were reported

B) in **Mendelian susceptibility to mycobacterial diseases** novel mutation, clinical and immunopathological features, and the utility of IFNy were described

C) in **sepsis due to Staphylococcus aureus, IL-6 autoantibodies** were identified as the cause of immune failure with systemic consequence for the first time

D) in the novel **Paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2** epidemiological observations, predictors of disease severity, management guidelines were established, and immune pathologic mechanisms, such as self-reactive B cell pathology, were identified

The applicant contributed to the results by establishing the hypotheses, designing the study protocols, performing the data collections and analyses, and managing the patients. In parallel, patient cohorts were established, national and international cooperations developed. During the doctoral study, the author actively presented in several national and international conferences.

Importantly, the findings were, in several cases, translated directly into the patients' clinical management. Our endeavours enabled risk stratifications, individualized management strategies, including avoidance behaviour, targeted therapeutic and prophylactic medications, procedures, and genetic counselling.

LITERATURE REFERENCES

1. Abadom, Tochukwu Raphael, Adrian D Smith, Stefano Tempia, Shabir A Madhi, Cheryl Cohen, and Adam L Cohen. 2016. "Risk Factors Associated with Hospitalisation for Influenza-Associated Severe Acute Respiratory Illness in South Africa: A Case-Population Study." *Vaccine* 34 (46): 5649–55.
2. Achkar, Jacqueline M, John Chan, and Arturo Casadevall. 2015. "B Cells and Antibodies in the Defense against Mycobacterium Tuberculosis Infection." *Immunological Reviews* 264 (1): 167–81.
3. Agarwal, Sarika, Viviana P Ferreira, Claudio Cortes, Michael K Pangburn, Peter A Rice, and Sanjay Ram. 2010. "An Evaluation of the Role of Properdin in Alternative Pathway Activation on Neisseria Meningitidis and Neisseria Gonorrhoeae." *The Journal of Immunology* 185 (1): 507–16.
4. Alandijany, Thamir. 2019. "Host Intrinsic and Innate Intracellular Immunity During Herpes Simplex Virus Type 1 (HSV-1) Infection." *Frontiers in Microbiology* 10 (November): 1–20.
5. Alberts B, Johnson A, Lewis J. 2002. "The Adaptive Immune System." In *Molecular Biology of the Cell.*, 4th ed.
6. Andersen, Line Lykke, Nanna Mørk, Line S Reinert, Emil Kofod-Olsen, Ryo Narita, Sofie E Jørgensen, Kristian A Skipper, et al. 2015. "Functional IRF3 Deficiency in a Patient with Herpes Simplex Encephalitis." *Journal of Experimental Medicine* 212 (9): 1371–79.
7. Arduino, Paolo G, and Stephen R Porter. 2008. "Herpes Simplex Virus Type 1 Infection: Overview on Relevant Clinico-Pathological Features*." *Journal of Oral Pathology & Medicine* 37 (2): 107–21.
8. Arenzana-Seisdedos, Fernando, and Marc Parmentier. 2006. "Genetics of Resistance to HIV Infection: Role of Co-Receptors and Co-Receptor Ligands." *Seminars in Immunology* 18 (6): 387–403.
9. Asano, Takaki, Bertrand Boisson, Fanny Onodi, Daniela Matuozzo, Marcela Moncada-Velez, Majistor Raj Luxman Maglorius Renkilaraj, Peng Zhang, et al. 2021. "X-Linked Recessive TLR7 Deficiency in ~1% of Men under 60 Years Old with Life-Threatening COVID-19." *Science Immunology* 6 (62): eabl4348.
10. Averbuch, Diana, Ariane Chapgier, Stéphanie Boisson-Dupuis, Jean-Laurent Casanova, and Dan Engelhard. 2011. "The Clinical Spectrum Of Patients With Deficiency Of Signal Transducer And Activator Of Transcription-1." *The Pediatric Infectious Disease Journal* 30 (4).
11. Báez-Gutierrez, Nerea, and Héctor Rodríguez-Ramallo. 2021. "Candidiasis in Patients Treated with Interleukin-17 Inhibitors." *Indian Journal of Pharmacology* 53 (3): 244–45.
12. Bastard, Paul, Lindsey B. Rosen, Qian Zhang, Eleftherios Michailidis, Hans Heinrich Hoffmann, Yu Zhang, Karim Dorgham, et al. 2020. "Autoantibodies against Type I IFNs in Patients with Life-Threatening COVID-19." *Science* 370 (6515).
13. Bennett, Craig L, Jacinda Christie, Fred Ramsdell, Mary E Brunkow, Polly J Ferguson, Luke Whitesell, Thaddeus E Kelly, Frank T Saulsbury, Phillip F Chance, and Hans D Ochs. 2001. "The Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome (IPEX) Is Caused by Mutations of FOXP3." *Nature Genetics* 27 (1): 20–21.
14. Béziat, Vivien, Juan Li, Jian-Xin Lin, Cindy S Ma, Peng Li, Aziz Bousfiha, Isabelle Pellier, et al. 2018. "A Recessive Form of Hyper-IgE Syndrome by Disruption of ZNF341-Dependent STAT3 Transcription and Activity." *Science Immunology* 3 (24): eaat4956.
15. Bhattad, Sagar, Jeeson Unni, and Sonny Varkey. 2021. "MSMD in a 3-Generation Multiplex Kindred Due to Autosomal Dominant STAT1 Deficiency." *Journal of Clinical Immunology* 41 (1): 259–61.
16. Bieniasz, Paul D. 2004. "Intrinsic Immunity: A Front-Line Defense against Viral Attack." *Nature Immunology* 5 (11): 1109–15.
17. Bilbo, Staci D, Carina L Block, Jessica L Bolton, Richa Hanamsagar, and Phuong K Tran. 2018. "Beyond Infection - Maternal Immune Activation by Environmental Factors, Microglial Development, and Relevance for Autism Spectrum Disorders." *Experimental Neurology* 299 (Pt A): 241–51.
18. Bloomfield, Markéta, Havránková, Eva, Houšťková, Hana, Kabíček Pavle, Křepela, Karel, Šedivá Anna, and Bustamante Jacinta. 2016. "Vrozená Vnímavost k Mykobakteriálním Onemocněním." *Česko-slovenská Pediatrie* 71 (7-8): 340-344.
19. Bloomfield, Markéta, Kanderová, Veronika, Paračková, Zuzana, Vrabcová, Petra, Svatoň, Michael, Froňková, Eva, Fejtková Martina et al. 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.
20. Bloos, Frank, Jürgen Held, Peter Schlattmann, Nicole Brillinger, Oliver Kurzai, Oliver A Cornely, and Daniel Thomas-Rüddel. 2018. "(1,3)-β-D-Glucan-Based Diagnosis of Invasive Candida Infection versus

Culture-Based Diagnosis in Patients with Sepsis and with an Increased Risk of Invasive Candida Infection (CandiSep): Study Protocol for a Randomized Controlled Trial." *Trials* 19 (1): 472.

21. Bluyssen, HansA.R., and David E Levy. 1997. "Stat2 Is a Transcriptional Activator That Requires Sequence-Specific Contacts Provided by Stat1 and P48 for Stable Interaction with DNA *." *Journal of Biological Chemistry* 272 (7): 4600–4605.
22. Bohlson, Suzanne, Strasser Jennifer A., Bower Jacquelyn J., and Schorey Jeffrey S. 2001. "Role of Complement in Mycobacterium Avium Pathogenesis: In Vivo and In Vitro Analyses of the Host Response to Infection in the Absence of Complement Component C3." *Infection and Immunity* 69 (12): 7729–35.
23. Boisson-Dupuis, Stephanie, Xiao Fei Kong, Satoshi Okada, Sophie Cypowyj, Anne Puel, Laurent Abel, and Jean Laurent Casanova. 2012. "Inborn Errors of Human STAT1: Allelic Heterogeneity Governs the Diversity of Immunological and Infectious Phenotypes." *Current Opinion in Immunology*.
24. Braubach, Peter, Torsten Lippmann, Didier Raoult, Jean Christophe Lagier, Ioannis Anagnostopoulos, Steffen Zender, Florian Peter Länger, Hans Heinrich Kreipe, Mark Philipp Kühnel, and Danny Jonigk. 2017. "Fluorescence in Situ Hybridization for Diagnosis of Whipple's Disease in Formalin-Fixed Paraffin-Embedded Tissue." *Frontiers in Medicine* 4 (JUN).
25. Brown, Kevin E, Jonathan R Hibbs, Giorgio Gallinella, Stacie M Anderson, Elton D Lehman, Peggy McCarthy, and Neal S Young. 1994. "Resistance to Parvovirus B19 Infection Due to Lack of Virus Receptor (Erythrocyte P Antigen)." *New England Journal of Medicine* 330 (17): 1192–96.
26. Browne, Sarah K, Peter D Burbelo, Ploenchan Chetchotisakd, Yupin Suputtamongkol, Sasisopin Kiertiburanakul, Pamela A Shaw, Jennifer L Kirk, et al. 2012. "Adult-Onset Immunodeficiency in Thailand and Taiwan." *New England Journal of Medicine* 367 (8): 725–34.
27. Bruton, Ogden. 1952. "Agammaglobulinemia." *Pediatrics* 9 (6): 722–28.
28. Bucciol, Giorgia, Leen Moens, Barbara Bosch, Xavier Bossuyt, Jean Laurent Casanova, Anne Puel, and Isabelle Meyts. 2019. "Lessons Learned from the Study of Human Inborn Errors of Innate Immunity." *Journal of Allergy and Clinical Immunology*.
29. Bustamante, Jacinta. 2020. "Mendelian Susceptibility to Mycobacterial Disease: Recent Discoveries." *Human Genetics* 139 (6): 993–1000.
30. Bustamante, Jacinta, Stéphanie Boisson-Dupuis, Laurent Abel, and Jean-Laurent Casanova. 2014. "Mendelian Susceptibility to Mycobacterial Disease: Genetic, Immunological, and Clinical Features of Inborn Errors of IFN-γ Immunity." *Seminars in Immunology* 26 (6): 454–70.
31. Casanova, Jean-Laurent, and Laurent Abel. 2004. "The Human Model: A Genetic Dissection of Immunity to Infection in Natural Conditions." *Nature Reviews Immunology* 4 (1): 55–66.
32. Casanova, Jean-Laurent and Abel, Laurent. 2005. "Inborn Errors of Immunity to Infection : The Rule Rather than the Exception ." *Journal of Experimental Medicine* 202 (2): 197–201.
33. Casanova, Jean-Laurent, Mary Ellen Conley, Stephen J Seligman, Laurent Abel, and Luigi D Notarangelo. 2014. "Guidelines for Genetic Studies in Single Patients: Lessons from Primary Immunodeficiencies." *Journal of Experimental Medicine* 211 (11): 2137–49.
34. Casanova, Jean Laurent, Claire Fieschi, Jacinta Bustamante, Janine Reichenbach, Natasha Remus, Horst Von Bernuth, and Capucine Picard. 2005. "From Idiopathic Infectious Diseases to Novel Primary Immunodeficiencies." *Journal of Allergy and Clinical Immunology* 116 (2): 426–30.
35. Chantrain, C F, A Bruwier, B Brichard, V Largent, A Chapgier, J Feinberg, J-L Casanova, J-P Stalens, and C Vermylen. 2006. "Successful Hematopoietic Stem Cell Transplantation in a Child with Active Disseminated Mycobacterium Fortuitum Infection and Interferon-γ Receptor 1 Deficiency." *Bone Marrow Transplantation* 38 (1): 75–76.
36. Chapman, Stephen J, and Adrian V S Hill. 2012. "Human Genetic Susceptibility to Infectious Disease." *Nature Reviews Genetics* 13 (3): 175–88.
37. Chi, Xinxin, Wei Jin, Xiaohong Zhao, Tian Xie, Jing Shao, Xue Bai, Yu Jiang, Xiaohu Wang, and Chen Dong. 2022. "RORγt Expression in Mature TH17 Cells Safeguards Their Lineage Specification by Inhibiting Conversion to TH2 Cells." *Science Advances* 8 (34): eabn7774.
38. Chin, Kai Ling, Maria E Sarmiento, Nadine Alvarez-Cabrera, Mohd Nor Norazmi, and Armando Acosta. 2020. "Pulmonary Non-Tuberculous Mycobacterial Infections: Current State and Future Management." *European Journal of Clinical Microbiology & Infectious Diseases* 39 (5): 799–826.
39. Chin, Voon K, Tze Y Lee, Basir Rusliza, and Pei P Chong. 2016. "Dissecting Candida Albicans Infection from the Perspective of C. Albicans Virulence and Omics Approaches on Host–Pathogen Interaction: A Review." *International Journal of Molecular Sciences 17 (10):1643*.

40. Choi, Young Jin, Hwi Jun Kim, Hee Bong Shin, Hae Seon Nam, Sang Han Lee, Joon Soo Park, Kwi Sung Park, and Kyoung Ah Baek. 2012. "Evaluation of Peptide Nucleic Acid Probe-Based Real-Time PCR for Detection of Mycobacterium Tuberculosis Complex and Nontuberculous Mycobacteria in Respiratory Specimens." *Annals of Laboratory Medicine* 32 (4): 257–63.
41. Chou, Janet, Craig D Platt, Saddiq Habiballah, Alan A Nguyen, Megan Elkins, Sabrina Weeks, Zachary Peters, et al. 2021. "Mechanisms Underlying Genetic Susceptibility to Multisystem Inflammatory Syndrome in Children (MIS-C)." *Journal of Allergy and Clinical Immunology* 148 (3): 732-738.e1.
42. Ciancanelli, Michael J, Sarah X L Huang, Priya Luthra, Hannah Garner, Yuval Itan, Stefano Volpi, Fabien G Lafaille, et al. 2015. "Life-Threatening Influenza and Impaired Interferon Amplification in Human IRF7 Deficiency." *Science* 348 (6233): 448–53.
43. Clohisey, Sara, and John Kenneth Baillie. 2019. "Host Susceptibility to Severe Influenza A Virus Infection." *Critical Care* 23 (1): 303.
44. Courtois, G, and A Smahi. 2006. "NF-KB-Related Genetic Diseases." *Cell Death & Differentiation* 13 (5): 843–51.
45. Crow, Yanick 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.
46. Crow, Yanick J, and Nicolas Manel. 2015. "Aicardi–Goutières Syndrome and the Type I Interferonopathies." *Nature Reviews Immunology* 15 (7): 429–40.
47. Curtis, James, Yang Luo, Helen L Zenner, Delphine Cuchet-Lourenço, Changxin Wu, Kitty Lo, Mailis Maes, et al. 2015. "Susceptibility to Tuberculosis Is Associated with Variants in the ASAP1 Gene Encoding a Regulator of Dendritic Cell Migration." *Nature Genetics* 47 (5): 523–27.
48. Darrason, Marie. 2013. "Unifying Diseases through Common Genetic Mechanisms: The Example of the Genetic Theory of Infectious Diseases." *Theoretical Medicine and Bioethics* 34 (4): 327–44.
49. Decker, THOMAS, PAVEL Kovarik, and Andras Meinke. 1997. "GAS Elements: A Few Nucleotides with a Major Impact on Cytokine-Induced Gene Expression." *Journal of Interferon & Cytokine Research* 17 (3): 121–34.
50. Depner, Mark, Sebastian Fuchs, Jan Raabe, Natalie Frede, Cristina Glocker, Rainer Doffinger, Effrossyni Gkrania-Klotsas, et al. 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.
51. Deyà-Martínez, Angela, Rivière, Jaques G, Pérsio Roxo-Junior, Ramakers, Jan, Bloomfield Marketa, Paloma Guisado Hernandez, Pilar Blanco Lobo, et al. 2022. "Impact of JAK Inhibitors in Pediatric Patients with STAT1 Gain of Function (GOF) Mutations—10 Children and Review of the Literature." *Journal of Clinical Immunology* 42 (5): 1071–82.
52. Dietz, S M, D van Stijn, D Burgner, M Levin, I M Kuipers, B A Hutten, and T W Kuijpers. 2017. "Dissecting Kawasaki Disease: A State-of-the-Art Review." *European Journal of Pediatrics* 176 (8): 995–1009.
53. Dolečková, Věra, J Viklický, L Šula, and Dagmar Kubecová. 1977. "Fatal Generalized BCG Histiocytosis." *Tubercle* 58 (1): 13–18.
54. Dolezalova, Karolina and Gopfertova, Dana. 2021. "Ten Years' Experience with the Discontinuation of the Bacillus Calmette–Guérin Vaccination in the Czech Republic." *International Journal of Mycobacteriology* 10 (4): 193–98.
55. Dolezalova Karolina, Strachan Tomas, Matej Radoslav, Ricna Dita, Bloomfield, Marketa. 2022. "Manifestations of Cutaneous Mycobacterial Infections in Inborn Errors of IL-12, IL-23/IFNy Immunity." *European Journal of Dermatology.* 2022; 7;32(4):1-10..
56. Domer, Judith E. 1989. "Candida Cell Wall Mannan: A Polysaccharide with Diverse Immunologic Properties." *Critical Reviews in Microbiology* 17 (1): 33–51.
57. Dorman, Susan E, Capucine Picard, David Lammas, Klaus Heyne, Jaap T van Dissel, Richard Baretto, Sergio D Rosenzweig, et al. 2004. "Clinical Features of Dominant and Recessive Interferon γ Receptor 1 Deficiencies." *The Lancet* 364 (9451): 2113–21.
58. Drummond, Rebecca A, Shinobu Saijo, Yoichiro Iwakura, and Gordon D Brown. 2011. "The Role of Syk/CARD9 Coupled C-Type Lectins in Antifungal Immunity." *European Journal of Immunology* 41 (2): 276–81.
59. Duncan, Christopher J.A., and Sophie Hambleton. 2014. "Host Genetic Factors in Susceptibility to Mycobacterial Disease." *Clinical Medicine, Journal of the Royal College of Physicians of London* 14 (6): S17–21.
60. Dupuis, Stéphanie, Catherine Dargemont, Claire Fieschi, Nicolas Thomassin, Sergio Rosenzweig, Jeff

Harris, Steven M Holland, Robert D Schreiber, and Jean-Laurent Casanova. 2001. "Impairment of Mycobacterial But Not Viral Immunity by a Germline Human STAT1 Mutation." *Science* 293 (5528): 300–303.

61. Durandy, Anne, Sven Kracker, and Alain Fischer. 2013. "Primary Antibody Deficiencies." *Nature Reviews Immunology* 13 (7): 519–33.
62. Dvořáček C, Mores A, Neoral L. 1959. "BCG." *Rozhledy v Tuberkulose a v Nemocech Plicních.*, no. 19: 107–14.
63. Elsori, Deena H, Valentin P Yakubenko, Talat Roome, Praveena S Thiagarajan, Ashish Bhattacharjee, Satya P Yadav, and Martha K Cathcart. 2011. "Protein Kinase Cδ Is a Critical Component of Dectin-1 Signaling in Primary Human Monocytes." *Journal of Leukocyte Biology* 90 (3): 599–611.
64. Eski, Aykut, Gökçen Kartal Öztürk, Figen Gülen, Candan Çiçek, and Esen Demir. 2019. "Risk Factors for Influenza Virus Related Severe Lower Respiratory Tract Infection in Children." *The Pediatric Infectious Disease Journal* 38 (11).
65. Fang, Fang, Liang Zhao, Ming-jun Jiang, Yan Wang, and Qian-Qiu Wang. 2008. "Epidermodysplasia Verruciformis with Severe Hand and Foot Deformity Successfully Treated with Surgical Excision." *The International Journal of Surgical Reconstruction* 61 (3): 338–41.
66. Feinberg, Jacqueline, Claire Fieschi, Rainer Doffinger, Max Feinberg, Tony Leclerc, Stéphanie Boisson-Dupuis, Capucine Picard, et al. 2004. "Bacillus Calmette Guérin Triggers the IL-12/IFN-γ Axis by an IRAK-4- and NEMO-Dependent, Non-Cognate Interaction between Monocytes, NK, and T Lymphocytes." *European Journal of Immunology* 34 (11): 3276–84.
67. Ferguson, J S, and L S Schlesinger. 2000. "Pulmonary Surfactant in Innate Immunity and the Pathogenesis of Tuberculosis." *Tubercle and Lung Disease* 80 (4): 173–84.
68. Figueroa, J E, and P Densen. 1991. "Infectious Diseases Associated with Complement Deficiencies." *Clinical Microbiology Reviews* 4 (3): 359–95.
69. Forbes, Lisa R., Tiphanie P. Vogel, Megan A. Cooper, Johana Castro-Wagner, Edith Schussler, Katja G. Weinacht, Ashley S. Plant, et al. 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).
70. Fraga, Alexandra G, Ana Margarida Barbosa, Catarina M Ferreira, João Fevereiro, Jorge Pedrosa, and Egídio Torrado. 2018. "Immune-Evasion Strategies of Mycobacteria and Their Implications for the Protective Immune Response." *Current Issues in Molecular Biology (7)* .
71. Frey-Jakobs, Stefanie, Julia M Hartberger, Manfred Fliegauf, Claudia Bossen, Magdalena L Wehmeyer, Johanna C Neubauer, Alla Bulashevska, et al. 2018. "ZNF341 Controls STAT3 Expression and Thereby Immunocompetence." *Science Immunology* 3 (24): eaat4941.
72. Gazendam, Roel P, John L van Hamme, Anton T J Tool, Michel van Houdt, Paul J J H Verkuijlen, Martin Herbst, Johannes G Liese, et al. 2014. "Two Independent Killing Mechanisms of Candida Albicans by Human Neutrophils: Evidence from Innate Immunity Defects." *Blood* 124 (4): 590–97.
73. Gianella-Borradori, Athos, Luca Borradori, and Peter Späth. 1988. "C5 Deficiency and Meningitis in a Swiss Family." *Archives of Internal Medicine* 148 (3): 754.
74. Giovannozzi, Simone, Jonas Demeulemeester, Rik Schrijvers, and Rik Gijsbers. 2021. "Transcriptional Profiling of STAT1 Gain-of-Function Reveals Common and Mutation-Specific Fingerprints." *Frontiers in Immunology* 12 (February): 1–14.
75. Glaser, Carol A, Kathleen Winter, Kara DuBray, Kathleen Harriman, Timothy M Uyeki, James Sejvar, Sabrina Gilliam, and Janice K Louie. 2012. "A Population-Based Study of Neurologic Manifestations of Severe Influenza A(H1N1)Pdm09 in California." *Clinical Infectious Diseases* 55 (4): 514–20.
76. Glöckner, Andreas, Angela Steinbach, Jörg Janne Vehreschild, and Oliver A Cornely. 2009. "Treatment of Invasive Candidiasis with Echinocandins." *Mycoses* 52 (6): 476–86.
77. Gow, Neil A R, Frank L van de Veerdonk, Alistair J P Brown, and Mihai G Netea. 2012. "Candida Albicans Morphogenesis and Host Defence: Discriminating Invasion from Colonization." *Nature Reviews Microbiology* 10 (2): 112–22.
78. Griffith, Jason W, Caroline L Sokol, and Andrew D Luster. 2014. "Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity." *Annual Review of Immunology* 32 (1): 659–702.
79. Guérin, Antoine, Gaspard Kerner, Nico Marr, Janet G Markle, Florence Fenollar, Natalie Wong, Sabri Boughorbel, et al. 2018. "IRF4 Haploinsufficiency in a Family with Whipple's Disease." Edited by Jos W M van der Meer. *ELife* 7 (March): e32340.

80. Hadders, Michael A., Doryen Bubeck, Pietro Roversi, Svetlana Hakobyan, Federico Forneris, B. Paul Morgan, Michael K. Pangburn, Oscar Llorca, Susan M. Lea, and Piet Gros. 2012. "Assembly and Regulation of the Membrane Attack Complex Based on Structures of C5b6 and SC5b9." *Cell Reports* 1 (3): 200–207.
81. Henkle, Emily, and Kevin L Winthrop. 2015. "Nontuberculous Mycobacteria Infections in Immunosuppressed Hosts." *Clinics in Chest Medicine* 36 (1): 91–99.
82. Hermansen, Thomas Stig, Vibeke Østergaard Thomsen, Troels Lillebaek, and Pernille Ravn. 2014. "Non-Tuberculous Mycobacteria and the Performance of Interferon Gamma Release Assays in Denmark." *PLOS ONE* 9 (4): e93986.
83. Hernandez, Nicholas, Isabelle Melki, Huie Jing, Tanwir Habib, Susie S Y Huang, Jeffrey Danielson, Tomasz Kula, et al. 2018. "Life-Threatening Influenza Pneumonitis in a Child with Inherited IRF9 Deficiency." *Journal of Experimental Medicine* 215 (10): 2567–85.
84. Hershkovitz, Israel, Helen D Donoghue, David E Minnikin, Gurdyal S Besra, Oona Y-C. Lee, Angela M Gernaey, Ehud Galili, et al. 2008. "Detection and Molecular Characterization of 9000-Year-Old Mycobacterium Tuberculosis from a Neolithic Settlement in the Eastern Mediterranean." *PLOS ONE* 3 (10): e3426.
85. Hiller, Julia, Beate Hagl, Renate Effner, Anne Puel, Martin Schaller, Birgit Mascher, Stefanie Eyerich, et al. 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): 711–14.
86. Horvath, Curt M. 2000. "STAT Proteins and Transcriptional Responses to Extracellular Signals." *Trends in Biochemical Sciences* 25 (10): 496–502.
87. Jain, Ajay, Sabina Kaczanowska, and Eduardo Davila. 2014. "IL-1 Receptor-Associated Kinase Signaling and Its Role in Inflammation, Cancer Progression, and Therapy Resistance." *Frontiers in Immunology* 5 (NOV): 1–8.
88. Jalaledin, Ghanavi, Farnia Poopak, Farnia Parissa, and Velayati Ali Akbar. 2021. "The Role of Interferon-Gamma and Interferon-Gamma Receptor in Tuberculosis and Nontuberculous Mycobacterial Infections." *International Journal of Mycobacteriology* 10 (4): 349–57.
89. Jones, Makoto M, Kevin L Winthrop, Scott D Nelson, Scott L Duvall, Olga V Patterson, Kevin E Nechodom, Kimberly E Findley, Lewis J Radonovich Jr., Matthew H Samore, and Kevin P Fennelly. 2018. "Epidemiology of Nontuberculous Mycobacterial Infections in the U.S. Veterans Health Administration." *PLOS ONE* 13 (6): e0197976.
90. Jong, Sarah J. de, Elias Imahorn, Peter Itin, Jouni Uitto, Gérard Orth, Emmanuelle Jouanguy, Jean Laurent Casanova, and Bettina Burger. 2018. "Epidermodysplasia Verruciformis: Inborn Errors of Immunity to Human Beta-Papillomaviruses." *Frontiers in Microbiology* 9 (JUN).
91. Jouanguy, Emmanuelle, Frédéric Altare, Salma Lamhamedi, Patrick Revy, Jean-François Emile, Melanie Newport, Michael Levin, et al. 1996. "Interferon-γ –Receptor Deficiency in an Infant with Fatal Bacille Calmette–Guérin Infection." *New England Journal of Medicine* 335 (26): 1956–62.
92. Jouanguy, Emmanuelle, Salma Lamhamedi-Cherradi, David Lammas, Susan E Dorman, Marie-Claude Fondanèche, Stéphanie Dupuis, Rainer Döffinger, et al. 1999. "A Human IFNGR1 Small Deletion Hotspot Associated with Dominant Susceptibility to Mycobacterial Infection." *Nature Genetics* 21 (4):
93. Kampmann, Beate, Cheryl Hemingway, Alick Stephens, Robert Davidson, Anna Goodsall, Suzanne Anderson, Mark Nicol, et al. 2005. "Acquired Predisposition to Mycobacterial Disease Due to Autoantibodies to IFN-γ." *The Journal of Clinical Investigation* 115 (9): 2480–88.
94. Kappelman, John, Mehmet Cihat Alçiçek, Nizamettin Kazancı, Michael Schultz, Mehmet Özkul, and Şevket Şen. 2008. "First Homo Erectus from Turkey and Implications for Migrations into Temperate Eurasia." *American Journal of Physical Anthropology* 135 (1): 110–16.
95. Kerner, Gaspard, Jérémie Rosain, Antoine Guérin, Ahmad Al-Khabaz, Carmen Oleaga-Quintas, Franck Rapaport, Michel J Massaad, et al. 2020. "Inherited Human IFN-γ Deficiency Underlies Mycobacterial Disease." *The Journal of Clinical Investigation* 130 (6): 3158–71.
96. Khan, Taj Ali, Kalsoom Kalsoom, Asif Iqbal, Huma Asif, Hazir Rahman, Syed Omar Farooq, Hassan Naveed, et al. 2016. "A Novel Missense Mutation in the NADPH Binding Domain of CYBB Abolishes the NADPH Oxidase Activity in a Male Patient with Increased Susceptibility to Infections." *Microbial Pathogenesis* 100: 163–69.
97. Khandelwal, Shreya, and Widhi Dubey. 2020. "Overview of Mycobacterium: A Review." *European Journal of Molecular & Clinical Medicine* 7 (11): 6198–6213.
98. Kiykim, Ayca, Louis Marie Charbonnier, Arzu Akcay, Elif Karakoc-Aydiner, Ahmet Ozen, Gulyuz Ozturk,

Talal A Chatila, and Safa Baris. 2019. "Hematopoietic Stem Cell Transplantation in Patients with Heterozygous STAT1 Gain-of-Function Mutation." *Journal of Clinical Immunology* 39 (1): 37–44.
99. Koch, Anastasia, and Valerie Mizrahi. 2018. "Mycobacterium Tuberculosis</Em>." *Trends in Microbiology* 26 (6): 555–56.
100. Kojima, Takeshi, Takahiko Horiuchi, H Nishizaka, Yasuo Fukumori, Tetsuki Amano, Kohei Nagasawa, Yoshiyuki Niho, and Kenshi Hayashi. 1998. "Genetic Basis of Human Complement C8 Alpha-Gamma Deficiency." *Journal of Immunology (Baltimore, Md. : 1950)* 161 (7): 3762–66.
101. Krismer, Bernhard, Christopher Weidenmaier, Alexander Zipperer, and Andreas Peschel. 2017. "The Commensal Lifestyle of Staphylococcus Aureus and Its Interactions with the Nasal Microbiota." *Nature Reviews Microbiology* 15 (11): 675–87.
102. Ku, Chen Lung, Chih Yu Chi, Horst von Bernuth, and Rainer Doffinger. 2020. "Autoantibodies against Cytokines: Phenocopies of Primary Immunodeficiencies?" *Human Genetics* 139 (6–7): 783–94.
103. Ku, Cheng-Lung, Horst von Bernuth, Capucine Picard, Shen-Ying Zhang, Huey-Hsuan Chang, Kun Yang, Maya Chrabieh, et al. 2007. "Selective Predisposition to Bacterial Infections in IRAK-4–Deficient Children: IRAK-4–Dependent TLRs Are Otherwise Redundant in Protective Immunity ." *Journal of Experimental Medicine* 204 (10): 2407–22.
104. Ku, Cheng-Lung, Capucine Picard, Melinda Erdös, Axel Jeurissen, Jacinta Bustamante, Anne Puel, Horst von Bernuth, et al. 2007. "IRAK4 and NEMO Mutations in Otherwise Healthy Children with Recurrent Invasive Pneumococcal Disease." *Journal of Medical Genetics* 44 (1): 16–23.
105. Lamb, Ruth C, and Goutam Dawn. 2014. "Cutaneous Non-Tuberculous Mycobacterial Infections." *International Journal of Dermatology* 53 (10): 1197–1204.
106. Lammas, D A, E De Heer, J D Edgar, V Novelli, A Ben-Smith, R Baretto, P Drysdale, et al. 2002. "Heterogeneity in the Granulomatous Response to Mycobacterial Infection in Patients with Defined Genetic Mutations in the Interleukin 12-Dependent Interferon-Gamma Production Pathway." *International Journal of Experimental Pathology* 83 (1): 1–20.
107. Lee, Pui Y, Craig D Platt, Sabrina Weeks, Rachael F Grace, George Maher, Kasey Gauthier, Sridevi Devana, et al. 2020. "Immune Dysregulation and Multisystem Inflammatory Syndrome in Children (MIS-C) in Individuals with Haploinsufficiency of SOCS1. *Journal of Allergy and Clinical Immunology* 146 (5): 1194-1200.e1.
108. Lee, Wen-I., Jing-Long Huang, Ting-Shu Wu, Ming-Hsun Lee, I.-Jung Chen, Kuang-Hiu Yu, Chien-Ying Liu, et al. 2013. "Patients with Inhibitory and Neutralizing Auto-Antibodies to Interferon-γ Resemble the Sporadic Adult-Onset Phenotype of Mendelian Susceptibility to Mycobacterial Disease (MSMD) Lacking Bacille Calmette–Guerin (BCG)-Induced Diseases." *Immunobiology* 218 (5): 762–71.
109. Leiding, Jennifer W, Satoshi Okada, David Hagin, Mario Abinun, Anna Shcherbina, Dmitry N Balashov, Vy H D Kim, et al. 2017. "Hematopoietic Stem Cell Transplantation in Patients with Gain-of-Function Signal Transducer and Activator of Transcription 1 Mutations." *Journal of Allergy and Clinical Immunology*.
110. Lewandowsky, F, and W Lutz. 1922. "Ein Fall Einer Bisher Nicht Beschriebenen Hauterkrankung (Epidermodysplasia Verruciformis)." *Archiv Für Dermatologie Und Syphilis* 141 (2): 193–203.
111. Lewis, Lisa A, and Sanjay Ram. 2014. "Meningococcal Disease and the Complement System." *Virulence* 5 (1): 98–126.
112. Li, Danyang, and Minghua Wu. 2021. "Pattern Recognition Receptors in Health and Diseases." *Signal Transduction and Targeted Therapy* 6 (1): 291..
113. Li, Yong, Yujiong Wang, and Xiaoming Liu. 2012. "The Role of Airway Epithelial Cells in Response to Mycobacteria Infection." Edited by S Sozzani. *Clinical and Developmental Immunology* 2012: 791392.
114. Lim, Hye Kyung, Sarah X L Huang, Jie Chen, Gaspard Kerner, Olivier Gilliaux, Paul Bastard, Kerry Dobbs, et al. 2019. "Severe Influenza Pneumonitis in Children with Inherited TLR3 Deficiency." *Journal of Experimental Medicine* 216 (9): 2038–56.
115. Lindesmith, Lisa, Christine Moe, Severine Marionneau, Nathalie Ruvoen, Xi Jiang, Lauren Lindblad, Paul Stewart, Jacques LePendu, and Ralph Baric. 2003. "Human Susceptibility and Resistance to Norwalk Virus Infection." *Nature Medicine* 9 (5): 548–53.
116. Liu, Luyan, Satoshi Okada, Xiao-Fei Kong, Alexandra Y Kreins, Sophie Cypowyj, Avinash Abhyankar, Julie Toubiana, et al. 2011. "Gain-of-Function Human STAT1 Mutations Impair IL-17 Immunity and Underlie Chronic Mucocutaneous Candidiasis." *The Journal of Experimental Medicine* 208 (8): 1635–48.
117. Liu, Rong, William A. Paxton, Sunny Choe, Daniel Ceradini, Scott R. Martin, Richard Horuk, Marcy E.

MacDonald, Heidi Stuhlmann, Richard A. Koup, and Nathaniel R. Landau. 1996. "Homozygous Defect in HIV-1 Coreceptor Accounts for Resistance of Some Multiply-Exposed Individuals to HIV-1 Infection." *Cell* 86 (3): 367–77.

118. Liu, Wei, and Dong Lai Ma. 2020. "Severe Epidermodysplasia Verruciformis." *Mayo Clinic Proceedings* 95 (9): 2035.
119. Liu, Zhenxing, Mi Zhou, Chao Yuan, Zhengyi Ni, Wenqiang Liu, Yang Tan, Dazhi Zhang, et al. 2022. "Two Novel STAT1 Mutations Cause Mendelian Susceptibility to Mycobacterial Disease." *Biochemical and Biophysical Research Communications* 591: 124–29.
120. Lockhart, Shawn R, Kizee A Etienne, Snigdha Vallabhaneni, Joveria Farooqi, Anuradha Chowdhary, Nelesh P Govender, Arnaldo Lopes Colombo, et al. 2017. "Simultaneous Emergence of Multidrug-Resistant Candida Auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses." *Clinical Infectious Diseases* 64 (2): 134–40.
121. López-Martínez, Rubén. 2010. "Candidosis, a New Challenge." *Clinics in Dermatology* 28 (2): 178–84.
122. López-Varela, Elisa, Alberto L García-Basteiro, Begoña Santiago, Dirk Wagner, Jakko van Ingen, and Beate Kampmann. 2015. "Non-Tuberculous Mycobacteria in Children: Muddying the Waters of Tuberculosis Diagnosis." *The Lancet Respiratory Medicine* 3 (3): 244–56.
123. Ma, Cindy S, Gary Y J Chew, Nicholas Simpson, Archana Priyadarshi, Melanie Wong, Bodo Grimbacher, David A Fulcher, Stuart G Tangye, and Matthew C Cook. 2008. "Deficiency of Th17 Cells in Hyper IgE Syndrome Due to Mutations in STAT3 ." *Journal of Experimental Medicine* 205 (7): 1551–57.
124. McCullers, Jonathan A. 2014. "The Co-Pathogenesis of Influenza Viruses with Bacteria in the Lung." *Nature Reviews Microbiology* 12 (4): 252–62.
125. Medzhitov, Ruslan, and Charles Janeway. 2000. "Innate Immunity." *New England Journal of Medicine* 343 (5): 338–44.
126. Millsop, Jillian W, and Nasim Fazel. 2016. "Oral Candidiasis." *Clinics in Dermatology* 34 (4): 487–94.
127. Mizoguchi, Yoko, and Satoshi Okada. 2021. "Inborn Errors of STAT1 Immunity." *Current Opinion in Immunology* 72: 59–64.
128. Mogensen, Trine H. 2009. "Pathogen Recognition and Inflammatory Signaling in Innate Immune Defenses." *Clinical Microbiology Reviews* 22 (2): 240–73.
129. Muderris, T, S Kaya, B Ormen, A Aksoy Gokmen, C Varer Akpinar, and S Yurtsever Gul. 2020. "Mortality and Risk Factor Analysis for Candida Blood Stream Infection: A Three-Year Retrospective Study." *Journal de Mycologie Médicale* 30 (3): 101008.
130. Nam, Sorim, and Jong-Seok Lim. 2016. "Essential Role of Interferon Regulatory Factor 4 (IRF4) in Immune Cell Development." *Archives of Pharmacal Research* 39 (11): 1548–55.
131. Nanki, Toshihiro, Ikumi Onoue, Kenji Nagasaka, Aiko Takayasu, Masashi Ebisawa, Tadashi Hosoya, Toshizumi Shirai, et al. 2013. "Suppression of Elevations in Serum C Reactive Protein Levels by Anti-IL-6 Autoantibodies in Two Patients with Severe Bacterial Infections." *Annals of the Rheumatic Diseases*.
132. Netea, Mihai G, Leo A B Joosten, Jos W M van der Meer, Bart-Jan Kullberg, and Frank L van de Veerdonk. 2015. "Immune Defence against Candida Fungal Infections." *Nature Reviews Immunology* 15 (10): 630–42.
133. Nguyen, Mai T T, Jan Pødenphant, and Pernille Ravn. 2013. "Three Cases of Severely Disseminated Staphylococcus Aureus Infection in Patients Treated with Tocilizumab." *Case Reports* 2013.
134. Nicolas, Radomski, Cambau Emmanuelle, Moulin Laurent, Haenn Sophie, Moilleron Régis, and Lucas Françoise S. 2010. "Comparison of Culture Methods for Isolation of Nontuberculous Mycobacteria from Surface Waters." *Applied and Environmental Microbiology* 76 (11): 3514–20.
135. Nicolle, C. 1937. *Destin Des Maladies Infectieuses*. Alcan, Paris.
136. Noma, Kosuke, Yoko Mizoguchi, Miyuki Tsumura, and Satoshi Okada. 2022. "Mendelian Susceptibility to Mycobacterial Diseases: State of the Art." *Clinical Microbiology and Infection* S1198-743X(22)00143-4.
137. Notarangelo, Luigi, Jean Laurent Casanova, Mary Ellen Conley, Helen Chapel, Alain Fischer, Jennifer Puck, Chaim Roifman, Reinhard Seger, and Raif S. Geha. 2006. "Primary Immunodeficiency Diseases: An Update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee Meeting in Budapest, 2005." *The Journal of Allergy and Clinical Immunology* 117 (4): 883–96.
138. Nürnberger, W, H Pietsch, R Seger, T Bufon, and V Wahn. 1989. "Familial Deficiency of the Seventh Component of Complement Associated with Recurrent Meningococcal Infections." *European Journal*

of Pediatrics 148 (8): 758–60.
139. Ochs, Hans D, and Walter H Hitzig. 2012. "History of Primary Immunodeficiency Diseases." *Current Opinion in Allergy and Clinical Immunology* 12 (6).
140. Okada, S., J. G. Markle, E. K. Deenick, F. Mele, D. Averbuch, M. Lagos, M. Alzahrani, et al. 2015. "Impairment of Immunity to Candida and Mycobacterium in Humans with Bi-Allelic RORC Mutations." *Science* 349 (6248): 606–13.
141. Okada, Satoshi, Takaki Asano, Kunihiko Moriya, Stephanie Boisson-Dupuis, Masao Kobayashi, Jean Laurent Casanova, and Anne Puel. 2020. "Human STAT1 Gain-of-Function Heterozygous Mutations: Chronic Mucocutaneous Candidiasis and Type I Interferonopathy." *Journal of Clinical Immunology* 40 (8): 1065–81.
142. Olbrich, Peter, Maria Teresa Martínez-Saavedra, José Maria Perez-Hurtado, Cristina Sanchez, Berta Sanchez, Caroline Deswarte, Ignacio Obando, et al. 2015. "Diagnostic and Therapeutic Challenges in a Child with Complete Interferon-γ Receptor 1 Deficiency." *Pediatric Blood & Cancer* 62 (11): 2036–39.
143. Osamu Hirata, Satoshi Okada, Miyuki Tsumura, Reiko Kagawa, Mizuka Miki, Hiroshi Kawaguchi, Kazuhiro Nakamura, et al. 2013. "Heterozygosity for the Y701C STAT1 Mutation in a Multiplex Kindred with Multifocal Osteomyelitis." *Haematologica* 98 (10 SE-Articles): 1641–49.
144. Ovadia, Adi, Nigel Sharfe, Cynthia Hawkins, Suzanne Laughlin, and Chaim M Roifman. 2018. "Two Different STAT1 Gain-of-Function Mutations Lead to Diverse IFN-γ-Mediated Gene Expression." *Npj Genomic Medicine* 3 (1): 23.
145. Park, Bonggoo, and George Y Liu. 2020. "Staphylococcus Aureus and Hyper-IgE Syndrome." *International Journal of Molecular Sciences* 21 (23).
146. Pestka, Sidney, Christopher D Krause, and Mark R Walter. 2004. "Interferons, Interferon-like Cytokines, and Their Receptors." *Immunological Reviews* 202 (1): 8–32.
147. Picard, Capucine, Jean-Laurent Casanova, and Laurent Abel. 2006. "Mendelian Traits That Confer Predisposition or Resistance to Specific Infections in Humans." *Current Opinion in Immunology* 18 (4): 383–90.
148. Picard, Capucine, Jean-Laurent Casanova, and Anne Puel. 2011. "Infectious Diseases in Patients with IRAK-4, MyD88, NEMO, or IκBα Deficiency." *Clinical Microbiology Reviews* 24 (3): 490–97.
149. Picard, Capucine, Anne Puel, Marion Bonnet, Cheng-Lung Ku, Jacinta Bustamante, Kun Yang, Claire Soudais, et al. 2003. "Pyogenic Bacterial Infections in Humans with IRAK-4 Deficiency." *Science* 299 (5615): 2076–79.
150. Piskorska, Katarzyna, Magdalena Sikora, Marlena Gołaś, Maria Dąbkowska, Leszek Pączek, and Ewa Swoboda-Kopeć. 2014. "Invasive Candidiasis Serological Diagnosis in Solid Organ Transplant Recipients." *Central European Journal of Immunology* 39 (2): 187–92.
151. Plantinga, Theo S, Melissa D Johnson, William K Scott, Leo A B Joosten, Jos W M van der Meer, John R Perfect, Bart Jan Kullberg, and Mihai G Netea. 2012. "Human Genetic Susceptibility to Candida Infections." *Medical Mycology* 50 (8): 785–94.
152. Porvaznik, I, I Solovič, and J Mokrý. 2017. "Non-Tuberculous Mycobacteria: Classification, Diagnostics, and Therapy BT - Respiratory Treatment and Prevention." Cham: Springer International Publishing 19–25.
153. Puel, Anne. 2020. "Human Inborn Errors of Immunity Underlying Superficial or Invasive Candidiasis." *Human Genetics* 139 (6): 1011–22.
154. Puel, Anne, Sophie Cypowyj, Jacinta Bustamante, Jill F. Wright, Luyan Liu, Hye Kyung Lim, Mélanie Migaud, et al. 2011a. "Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity." *Science* 332 (6025): 65–68.
155. Puel, Anne, Sophie Cypowyj, Jacinta Bustamante, Jill F Wright, Luyan Liu, Hye Kyung Lim, Mélanie Migaud, et al. 2011b. "Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity." *Science* 332 (6025): 65–68.
156. Puel, Anne, Rainer Döffinger, Angels Natividad, Maya Chrabieh, Gabriela Barcenas-Morales, Capucine Picard, Aurélie Cobat, et al. 2010. "Autoantibodies against IL-17A, IL-17F, and IL-22 in Patients with Chronic Mucocutaneous Candidiasis and Autoimmune Polyendocrine Syndrome Type I." *Journal of Experimental Medicine* 207 (2): 291–97.
157. Puel, Anne, Capucine Picard, Mathie Lorrot, Charlotte Pons, Maya Chrabieh, Lazaro Lorenzo, Maria Mamani-Matsuda, Emmanuelle Jouanguy, Dominique Gendrel, and Jean-Laurent Casanova. 2008. "Recurrent Staphylococcal Cellulitis and Subcutaneous Abscesses in a Child with Autoantibodies against IL-6." *The Journal of Immunology*.

158. Ramoz, Nicolas, Luis-Alfredo Rueda, Bakar Bouadjar, Luz-Stella Montoya, Gérard Orth, and Michel Favre. 2002. "Mutations in Two Adjacent Novel Genes Are Associated with Epidermodysplasia Verruciformis." *Nature Genetics* 32 (4): 579–81.
159. Raoult, Didier, Marie L Birg, Bernard La Scola, Pierre E Fournier, Maryse Enea, Hubert Lepidi, Veronique Roux, et al. 2000. "Cultivation of the Bacillus of Whipple's Disease." *New England Journal of Medicine* 342 (9): 620–25.
160. Reichenbach, Janine, Sergio Rosenzweig, Rainer Döffinger, Stéphanie Dupuis, Steven M Holland, and Jean-Laurent Casanova. 2001. "Mycobacterial Diseases in Primary Immunodeficiencies." *Current Opinion in Allergy and Clinical Immunology* 1 (6).
161. Riphagen, Shelley, Xabier Gomez, Carmen Gonzalez-Martinez, Nick Wilkinson, and Paraskevi Theocharis. 2020. "Hyperinflammatory Shock in Children during COVID-19 Pandemic." *The Lancet* 395 (10237): 1607–8.
162. Romberg, Neil, Henner Morbach, Monica G Lawrence, Sanghyun Kim, Insoo Kang, Steven M Holland, Joshua D Milner, and Eric Meffre. 2017. "Gain-of-Function STAT1</Em> Mutations Are Associated with PD-L1 Overexpression and a Defect in B-Cell Survival." *Journal of Allergy and Clinical Immunology* 131 (6): 1691–93.
163. Rottman, Martin, Claire Soudais, Guillaume Vogt, Laurent Renia, Jean-François Emile, Hélène Decaluwe, Jean-Louis Gaillard, and Jean-Laurent Casanova. 2008. "IFN-γ Mediates the Rejection of Haematopoietic Stem Cells in IFN-ΓR1-Deficient Hosts." *PLOS Medicine* 5 (1): e26.
164. Russell, David G. 2007. "Who Puts the Tubercle in Tuberculosis?" *Nature Reviews Microbiology* 5 (1): 39–47.
165. Saffert, Ryan T, and Robert F Kalejta. 2006. "Inactivating a Cellular Intrinsic Immune Defense Mediated by Daxx Is the Mechanism through Which the Human Cytomegalovirus Pp71 Protein Stimulates Viral Immediate-Early Gene Expression." *Journal of Virology* 80 (8): 3863–71.
166. Sancho-shimizu, Vanessa, and Petter Brodin. 2021. "SARS-CoV-2 – Related MIS-C : A Key to the Viral and Genetic Causes of Kawasaki Disease ?" 218 (6).
167. Schelenz, Silke. 2008. "Management of Candidiasis in the Intensive Care Unit." *Journal of Antimicrobial Chemotherapy* 61 (suppl_1): i31–34.
168. Schindler, Christian, David E Levy, and Thomas Decker. 2007. "JAK-STAT Signaling: From Interferons to Cytokines*." *Journal of Biological Chemistry* 282 (28): 20059–63.
169. Shah, Kabeer K, Bobbi S Pritt, and Mariam P Alexander. 2017. "Histopathologic Review of Granulomatous Inflammation." *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases* 7: 1–12.
170. Shawn, Lockhart, Iqbal Naureen, Cleveland Angela A., Farley Monica M., Harrison Lee H., Bolden Carol B., Baughman Wendy, et al. 2012. "Species Identification and Antifungal Susceptibility Testing of Candida Bloodstream Isolates from Population-Based Surveillance Studies in Two U.S. Cities from 2008 to 2011." *Journal of Clinical Microbiology* 50 (11): 3435–42.
171. Sheehy, Ann M, Nathan C Gaddis, Jonathan D Choi, and Michael H Malim. 2002. "Isolation of a Human Gene That Inhibits HIV-1 Infection and Is Suppressed by the Viral Vif Protein." *Nature* 418 (6898): 646–50.
172. Sologuren, Ithaisa, María Teresa Martínez-Saavedra, Jordi Solé-Violán, Edgar de Borges de Oliveira Jr, Eva Betancor, Inmaculada Casas, Carmen Oleaga-Quintas, et al. 2018. "Lethal Influenza in Two Related Adults with Inherited GATA2 Deficiency." *Journal of Clinical Immunology* 38 (4): 513–26.
173. Spencer, Sarah, Sevgi Köstel Bal, William Egner, Hana Lango Allen, Syed I Raza, Chi A Ma, Meltem Gürel, et al. 2019. "Loss of the Interleukin-6 Receptor Causes Immunodeficiency, Atopy, and Abnormal Inflammatory Responses." *The Journal of Experimental Medicine* 216 (9): 1986 LP – 1998.
174. Stremlau, Matthew, Christopher M Owens, Michel J Perron, Michael Kiessling, Patrick Autissier, and Joseph Sodroski. 2004. "The Cytoplasmic Body Component TRIM5α Restricts HIV-1 Infection in Old World Monkeys." *Nature* 427 (6977): 848–53.
175. Sveinbjornsson, Gardar, Daniel F Gudbjartsson, Bjarni V Halldorsson, Karl G Kristinsson, Magnus Gottfredsson, Jeffrey C Barrett, Larus J Gudmundsson, et al. 2016. "HLA Class II Sequence Variants Influence Tuberculosis Risk in Populations of European Ancestry." *Nature Genetics* 48 (3): 318–22.
176. Swenson, Colin, Christa S. Zerbe, and Kevin Fennelly. 2018. "Host Variability in NTM Disease: Implications for Research Needs." *Frontiers in Microbiology* 9.
177. Tanaka, Toshio, Masashi Narazaki, and Tadamitsu Kishimoto. 2011. "Anti-Interleukin-6 Receptor Antibody, Tocilizumab, for the Treatment of Autoimmune Diseases." *FEBS Letters* 585 (23): 3699–

3709.
178. Tanaka, Toshio, Masashi Narazaki, and Tadamitsu Kishimoto. 2014. "Il-6 in Inflammation, Immunity, And Disease." *Cold Spring Harbor Perspectives in Biology*.
179. Tangye, Stuart G, Waleed Al-Herz, Aziz Bousfiha, Charlotte Cunningham-Rundles, Jose Luis Franco, Steven M Holland, Christoph Klein, et al. 2022. "Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee." *Journal of Clinical Immunology* 24;1-3.
180. Taur, Prasad D., Vijaya Gowri, Ambreen Abdulwahab Pandrowala, Vaishnavi V. Iyengar, Akshaya Chougule, Zainab Golwala, Shraddha Chandak, et al. 2021. "Clinical and Molecular Findings in Mendelian Susceptibility to Mycobacterial Diseases: Experience From India." *Frontiers in Immunology* 12 (February): 1–12.
181. Thomas-Rüddel, Daniel O, Peter Schlattmann, Mathias Pletz, Oliver Kurzai, and Frank Bloos. 2022. "Risk Factors for Invasive Candida</Em> Infection in Critically Ill Patients: A Systematic Review and Meta-Analysis." *CHEST* 161 (2): 345–55.
182. Thorven, Maria, Ammi Grahn, Kjell-Olof Hedlund, Hugo Johansson, Christer Wahlfrid, Göran Larson, and Lennart Svensson. 2005. "A Homozygous Nonsense Mutation (428G\&\#x2192;A) in the Human Secretor (*FUT2*) Gene Provides Resistance to Symptomatic Norovirus (GGII) Infections." *Journal of Virology* 79 (24): 15351–55.
183. Thye, Thorsten, Ellis Owusu-Dabo, Fredrik O Vannberg, Reinout van Crevel, James Curtis, Edhyana Sahiratmadja, Yanina Balabanova, et al. 2012. "Common Variants at 11p13 Are Associated with Susceptibility to Tuberculosis." *Nature Genetics* 44 (3): 257–59.
184. Thye, Thorsten, Fredrik O Vannberg, Sunny H Wong, Ellis Owusu-Dabo, Ivy Osei, John Gyapong, Giorgio Sirugo, et al. 2010. "Genome-Wide Association Analyses Identifies a Susceptibility Locus for Tuberculosis on Chromosome 18q11.2." *Nature Genetics* 42 (9): 739–41.
185. Toubiana, Julie, Satoshi Okada, Julia Hiller, Matias Oleastro, Macarena Lagos Gomez, Juan Carlos Aldave Becerra, Marie Ouachée-Chardin, et al. 2016. "Heterozygous STAT1 Gain-of-Function Mutations Underlie an Unexpectedly Broad Clinical Phenotype." *Blood* 127 (25): 3154–64.
186. Tournamille, Christophe, Yves Colin, Jean Pierre Cartron, and Caroline Le Van Kim. 1995. "Disruption of a GATA Motif in the Duffy Gene Promoter Abolishes Erythroid Gene Expression in Duffy–Negative Individuals." *Nature Genetics* 10 (2): 224–28.
187. Tsumura, Miyuki, Mizuka Miki, Yoko Mizoguchi, Osamu Hirata, Shiho Nishimura, Moe Tamaura, Reiko Kagawa, Seiichi Hayakawa, Masao Kobayashi, and Satoshi Okada. 2022. "Enhanced Osteoclastogenesis in Patients with MSMD Due to Impaired Response to IFN-γ" *Journal of Allergy and Clinical Immunology* 149 (1): 252-261.e6.
188. Tsumura, Miyuki, Satoshi Okada, Hidemasa Sakai, Shin'ichiro Yasunaga, Motoaki Ohtsubo, Takuji Murata, Hideto Obata, et al. 2012. "Dominant-Negative STAT1 SH2 Domain Mutations in Unrelated Patients with Mendelian Susceptibility to Mycobacterial Disease." *Human Mutation* 33 (9): 1377–87.
189. Uzel, Gulbu, Elizabeth P. Sampaio, Monica G. Lawrence, Amy P. Hsu, Mary Hackett, Morna J. Dorsey, Richard J. Noel, et al. 2013. "Dominant Gain-of-Function STAT1 Mutations in FOXP3 Wild-Type Immune Dysregulation-Polyendocrinopathy-Enteropathy-X-Linked-like Syndrome." *Journal of Allergy and Clinical Immunology* 131 (6).
190. Veerdonk, Frank L van de, Theo S Plantinga, Alexander Hoischen, Sanne P Smeekens, Leo A B Joosten, Christian Gilissen, Peer Arts, et al. 2011. "STAT1 Mutations in Autosomal Dominant Chronic Mucocutaneous Candidiasis." *New England Journal of Medicine* 365 (1): 54–61.
191. Vinh, Donald C. 2011. "Insights into Human Antifungal Immunity from Primary Immunodeficiencies." *The Lancet Infectious Diseases* 11 (10): 780–92.
192. Wang, Xiaowen, and Frank L van de Veerdonk. 2016. "When the Fight against Fungi Goes Wrong." *PLOS Pathogens* 12 (2): e1005400.
193. Wi, Yu Mi. 2019. "Treatment of Extrapulmonary Nontuberculous Mycobacterial Diseases." *Infect Chemother* 51 (3): 245–55.
194. Wolff, Dominik, Sarah Nee, Natalie Sandy Hickey, and Michael Marschollek. 2021. "Risk Factors for Covid-19 Severity and Fatality: A Structured Literature Review." *Infection* 49 (1): 15–28.
195. World Health Organization. 2018. "WHO Seasonal Influenza." https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).
196. Xie, Yifan, Fenli Shao, Juan Lei, and Na Huang. 2022. "Case Report : A STAT1 Gain-of- Function Mutation Causes a Syndrome of Combined Immunode Fi Ciency , Autoimmunity and Pure Red Cell

Aplasia," no. August: 1–7.
197. Yan, Nan, and Zhijian J Chen. 2012. "Intrinsic Antiviral Immunity." *Nature Immunology* 13 (3): 214–22.
198. Zhang, Qian, Zhiyong Liu, Marcela Moncada-Velez, Jie Chen, Masato Ogishi, Benedetta Bigio, Rui Yang, et al. 2020. "Inborn Errors of Type I IFN Immunity in Patients with Life-Threatening COVID-19." *Science* 370 (6515).
199. Zhang, Shen-Ying, and Jean-Laurent Casanova. 2015. "Inborn Errors Underlying Herpes Simplex Encephalitis: From TLR3 to IRF3." *Journal of Experimental Medicine* 212 (9): 1342–43.
200. Zhang, Shen Ying, Nathaniel E. Clark, Catherine A. Freije, Elodie Pauwels, Allison J. Taggart, Satoshi Okada, Hanna Mandel, et al. 2018. "Inborn Errors of RNA Lariat Metabolism in Humans with Brainstem Viral Infection." *Cell* 172 (5): 952-965.e18.
201. Zhang, Wenjing, Xuemei Chen, Guodong Gao, Shubin Xing, Lina Zhou, Xuemei Tang, Xiaodong Zhao, and Yunfei An. 2021. "Clinical Relevance of Gain- and Loss-of-Function Germline Mutations in STAT1: A Systematic Review." *Frontiers in Immunology* 12 (March): 1–14.
202. Zheng, Jie, Frank L. van de Veerdonk, Katherine L. Crossland, Sanne P. Smeekens, Chun M. Chan, Tariq Al Shehri, Mario Abinun, et al. 2015. "Gain-of-Function STAT1 Mutations Impair STAT3 Activity in Patients with Chronic Mucocutaneous Candidiasis (CMC)." *European Journal of Immunology* 45 (10): 2834–46.
203. Zheng, Ruijuan, Zhiqiang Li, Fusheng He, Haipeng Liu, Jianhua Chen, Jiayu Chen, Xuefeng Xie, et al. 2018. "Genome-Wide Association Study Identifies Two Risk Loci for Tuberculosis in Han Chinese." *Nature Communications* 9 (1): 4072.
204. Zhou, Yunchun, Wei Mu, Jihua Zhang, Shi Wu Wen, and Smita Pakhale. 2022. "Global Prevalence of Non-Tuberculous Mycobacteria in Adults with Non-Cystic Fibrosis Bronchiectasis 2006–2021: A Systematic Review and Meta-Analysis." *BMJ Open* 12 (8): e055672.
205. Zimmerman, Ofer, Peter Olbrich, Alexandra F. Freeman, Lindsey B. Rosen, Gulbu Uzel, Christa S. Zerbe, Sergio D. Rosenzweig, et al. 2019. "STAT1 Gain-of-Function Mutations Cause High Total STAT1 Levels with Normal Dephosphorylation." *Frontiers in Immunology* 10 (JULY): 4–6.
206. Zimmerman, Ofer, Lindsey B. Rosen, Muthulekha Swamydas, Elise M.N. Ferre, Mukil Natarajan, Frank van de Veerdonk, Steven M. Holland, and Michail S. Lionakis. 2017. "Autoimmune Regulator Deficiency Results in a Decrease in STAT1 Levels in Human Monocytes." *Frontiers in Immunology* 8 (JUL): 1–8.
207. Zimmermann, Natalie, Verena Thormann, Bo Hu, Anne-Britta Köhler, Aki Imai-Matsushima, Camille Locht, Eusondia Arnett, et al. 2016. "Human Isotype-Dependent Inhibitory Antibody Responses against Mycobacterium Tuberculosis." *EMBO Molecular Medicine* 8 (11): 1325–39.

LIST OF PUBLICATIONS

PUBLICATIONS SUPPORTING THE THESIS

Publications with impact factor, first or last authorship of the applicant:

1. **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. Utility of Ruxolitinib in a Child with Chronic Mucocutaneous Candidiasis Caused by a Novel STAT1 Gain-of-Function Mutation. *J Clin Immunol.* 2018;38(5):589-601. (IF=4.85, Q1)

2. **Bloomfield M**, Parackova Z, Hanzlikova J, Lastovicka J, Sediva A. Immunogenicity and Safety of COVID-19 mRNA Vaccine in STAT1 GOF Patients. *J Clin Immunol.* 2022;42(2):266-269. (IF 8.32, Q1)

3. Parackova, Z*, **Bloomfield M***, Vrabcova P, Zentsova I, Klocperk A, Milota T, Svaton M, Casanova JL, Bustamante J, Fronkova E, Sediva A. 2019. Mutual alteration of NOD2-associated Blau syndrome and IFNγR1 deficiency. *J. Clin. Immunol.* 2020; 40:165–178.
*Authors contributed equally (IF=6.78, Q1)

4. Dolezalova K, Strachan T, Matej R, Ricna D, **Bloomfield M.** Manifestations of cutaneous mycobacterial infections in inborn errors of Il-12, Il-23/IFNy immunity. *European Journal of Dermatology*. 2022; 7;32(4):1-10. (IF 2.8, Q2)

5. **Bloomfield M***, Parackova Z*, Cabelova T, Pospisilova I, Kabicek P, Houstkova H, Sediva A. Anti-IL6 Autoantibodies in an Infant With CRP-Less Septic Shock. *Front. Immunol*. 2019;10: 1–6.
*Authors contributed equally (IF=5.05, Q1)

Publications with impact factor, authorship of the applicant:

6. Deyà-Martínez A, Rivière JG, Roxo-Junior P, Ramakers J, **Bloomfield M,** Guisado Hernandez P, Blanco Lobo P, Abu Jamra SR, Esteve-Sole A, Kanderova V, García-García A, Lopez-Corbeto M, Martinez Pomar N, Martín-Nalda A, Alsina L, Neth O, Olbrich P. Impact of JAK Inhibitors in Pediatric Patients with STAT1 Gain of Function (GOF) Mutations-10 Children and Review of the Literature. *J Clin Immunol.* 2022 Jul;42(5):1071-1082. (IF 8.32, Q1)

7. David J, Stara V, Hradsky O, Tuckova J, Slaba K, Jabandziev P, Sasek L, Huml M, Zidkova I, Pavlicek J, Palatova A, Klaskova E, Banszka K, Terifajova E, Vyhnanek R, **Bloomfield M,** Fingerhutova S, Dolezalova P, Prochazkova L, Chramostova G, Fencl F, Lebl J. Nationwide observational study of paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the Czech Republic. *Eur J Pediatr.* 2022 Aug 20:1–10. (IF 3.86, Q1)

8. Feleszko W, Okarska-Napierała M, Pauline Budding E, **Bloomfield M,** Sediva A, Bautista-Rodriguez C, Brough HA, Eigenmann PA, Eiwegger T, Eljaszewicz A, Eyerich S, Gomez-Casado C, Fraisse A, Janda J, Jiméeneéz-Saiz R, Kallinich T, Krohn IK, Mortz CG, Riggioni C, Sastre J, Sokolowska M, Strzelczyk Z, Untersmayr E, Tramper-Stranders G. Under review in *Allergy,* 2022. (IF 14.71, Q1)

Publications without impact factor:

9. Prucha M, Grombirikova H, Zdrahal P, **Bloomfield M,** Parackova Z, Freiberger T. Mendelian Susceptibility to Mycobacterial Disease: The First Case of a Diagnosed Adult Patient in the Czech Republic. *Case Reports Immunol.* 2020 Dec 19; 8836685. (without IF)

10. Klocperk A*, **Bloomfield M*,** Parackova Z, Aillot L, Fremuth L, Sasek L, David J, Fencl F, Skotniova A, Rejlova K, Magner M, Hrusak O, Sediva A. B cells, BAFF and interferons in MIS-C. *MedRxiv preprint*, version posted May 21, 2022. (without IF)
    *Authors contributed equally

OTHER PUBLICATIONS

1. **Bloomfield M**, Klocperk A, Zachova R, Milota T, Kanderova V, Sediva A. Natural Course of Activated Phosphoinositide 3-Kinase Delta Syndrome in Childhood and Adolescence. *Front Pediatr.* 2021 Jul 19;9:697706. (IF 3.42, Q2)

2. Fejtkova M, Sukova M, Hlozkova K, Skvarova Kramarzova K, Rackova M, Jakubec D, Bakardjieva M, **Bloomfield M**, Klocperk A, Parackova Z, Sediva A, Aluri J, Novakova M, Kalina T, Fronkova E, Hrusak O, Malcova H, Sedlacek P, Liba Z, Kudr M, Stary J, Cooper MA, Svaton M, Kanderova V. TLR8/TLR7 dysregulation due to a novel TLR8 mutation causes severe autoimmune hemolytic anemia and autoinflammation in identical twins. *Am J Hematol.* 2022 Mar 1;97(3):338-351. (IF 13.27, Q1)

4. Smetanova J, Milota T, Rataj M, **Bloomfield M**, Sediva A, Klocperk A. Accelerated Maturation, Exhaustion, and Senescence of T cells in 22q11.2 Deletion Syndrome. *J Clin Immunol.* 2022 Feb;42(2):274-285. (IF 8.32, Q1)

5. Kanderova V, Grombirikova H, Zentsova I, Reblova K, Klocperk A, Fejtkova M, **Bloomfield M**, Ravcukova B, Kalina T, Freiberger T, Sediva A. Lymphoproliferation, immunodeficiency and early-onset inflammatory bowel disease associated with a novel mutation in Caspase 8. *Haematologica.* 2019 Jan;104(1): e32-e34. (IF 7.12, Q1)

6. Mensa-Vilaró A, Bravo García-Morato M, de la Calle-Martin O, Franco-Jarava C, Martínez-Saavedra MT, González-Granado LI, González-Roca E, Fuster JL, Alsina L, Mutchinick OM, Balderrama-Rodríguez A, Ramos E, Modesto C, Mesa-Del-Castillo P, Ortego-Centeno N, Clemente D, Souto A, Palmou N, Remesal A, Leslie KS, Gómez de la Fuente E, Yadira Bravo Gallego L, Campistol JM, Dhouib NG, Bejaoui M, Dutra LA, Terreri MT, Mosquera C, González T, Cañellas J, García-Ruiz de Morales JM, Wouters CH, Bosque MT, Cham WT, Jiménez-Treviño S, de Inocencio J, **Bloomfield M**, Pérez de Diego R, Martínez-Pomar N, Rodríguez-Pena R, González-Santesteban C, Soler-Palacín P, Casals F, Yagüe J, Allende LM, Rodríguez-Gallego JC, Colobran R, Martínez-Martínez L, López-Granados E, Aróstegui JI. Unexpected relevant role of gene mosaicism in patients with primary immunodeficiency diseases. *J Allergy Clin Immunol.* 2019 Jan;143(1):359-368. (IF 10.23, Q1)

7. Klocperk A, Paračková Z, **Bloomfield M**, Rataj M, Pokorný J, Unger S, Warnatz K, Šedivá A. Follicular Helper T Cells in DiGeorge Syndrome. *Front Immunol.* 2018 Jul 23;9:1730. (IF 4.62, Q1)

8. Kralickova P, Milota T, Litzman J, Malkusova I, Jilek D, Petanova J, Vydlakova J, Zimulova A, Fronkova E, Svaton M, Kanderova V, **Bloomfield M**, Parackova Z, Klocperk A, Haviger J, Kalina T, Sediva A. CVID-Associated Tumors: Czech Nationwide Study Focused on Epidemiology, Immunology, and Genetic Background in a Cohort of Patients With CVID. *Front Immunol.* 2019 Jan 22;9:3135. (IF 4.96, Q1)

9. Chovancova Z, Kralickova P, Pejchalova A, **Bloomfield M**, Nechvatalova J, Vlkova M, Litzman J. Selective IgM Deficiency: Clinical and Laboratory Features of 17 Patients and a Review of the Literature. *J Clin Immunol.* 2017 Aug;37(6):559-574. (IF 4.23, Q1)

10. **Bloomfield M**, Pospisilova I, Cabelova T, Sediva A, Ibrahimova M, Borecka K, Magner M. Searching for COVID-19 Antibodies in Czech Children-A Needle in the Haystack. *Front Pediatr.* 2020 Nov 12;8:597736. (IF 3.42, Q2)

11. Parackova Z, Zentsova I, **Bloomfield M**, Vrabcova P, Smetanova J, Klocperk A, Mesežnikov G, Casas Mendez LF, Vymazal T, Sediva A. Disharmonic Inflammatory Signatures in COVID-19: Augmented Neutrophils' but Impaired Monocytes' and Dendritic Cells' Responsiveness. *Cells.* 2020 Sep 29;9(10):2206. (IF 6.6, Q2)

12. Parackova Z, **Bloomfield M**, Klocperk A, Sediva A. Neutrophils mediate Th17 promotion in COVID-19 patients. *J Leukoc Biol.* 2021 Jan;109(1):73-76. (IF 4.96, Q2)

13. Milota T, Sobotkova M, Smetanova J, **Bloomfield M**, Vydlakova J, Chovancova Z, Litzman J, Hakl R, Novak J, Malkusova I, Hanzlikova J, Jilek D, Hutyrova B, Novak V, Krcmova I, Sediva A, Kralickova P. Risk Factors for Severe COVID-19 and Hospital Admission in Patients with Inborn Errors of Immunity - Results from a Multicenter Nationwide Study. *Front Immunol.* 2022 Feb 28;13:835770. (IF 8.79, Q1)

14. Klocperk A, **Bloomfield M**, Parackova Z, Zentsova I, Vrabcova P, Balko J, Meseznikov G, Casas Mendez LF, Grandcourtova A, Sipek J, Tulach M, Zamecnik J, Vymazal T, Sediva A. Complex Immunometabolic Profiling Reveals the Activation of Cellular Immunity and Biliary Lesions in Patients with Severe COVID-19*. J Clin Med.* 2020 Sep 17;9(9):3000. (IF 4.24, Q2)

15. Zhang et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4570. (IF 47.73, Q1)

16. Bastard et al. Autoantibodies neutralizing type I IFNs are present in ~ 4% of uninfected individuals over 70 years and account for ~ 20% of COVID-19 deaths. Science Immunology. 2021 Aug 19;6(62):eabl4340. (IF 17.73, Q1)

17. Bastard et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4585. (IF 47.73, Q1)

18. Asano et al. X-linked recessive TLR7 deficiency in 1% of men under 60 years with life-threatening COVID-19. Science Immunology. 2021 Aug 19;6(62):eabl4348. (IF 17.73, Q1)

19. Sancho-Shimizu et al. SARS-CoV-2–related MIS-C: A key to the viral and genetic causes of Kawasaki disease? J Exp Med. 2021 Jun 7;218(6):e20210446 (IF 14.3, Q1)

APPENDIX I

LIST OF TABLES

LIST OF FIGURES

APPENDIX II-XI ATTACHED MANUSCRIPTS