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T lymfocyty jako klíčoví hráči v etiopatogenezi vrozených poruch imunity

T lymphocytes as key players in inborn errors of immunity

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Adam Klocperk

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IMMUNE SYSTEM IN THE 21ST CENTURY: THE INTERCONNECTED NETWORK OF THOUSANDS OF CELLS AND CYTOKINES, THE IMMUNE “BRAIN” SYSTEM

Scio me nihil scire

The immune system is a complex ecosystem of cells, soluble proteins, intercellular relationships, enzymatic cascades, positive and negative feedback loops, both deterministic and stochastic activation and inhibition thresholds, interspersed throughout the vast majority of the human body. Its activity and way of operation changes with host age, between locations in the body, depending on the condition of surrounding tissues, cytokine milieu, present cells and their ontological status, and can be affected by mood, endocrine and metabolic status and a myriad of other conditions. The complexity of its function and regulation networks is matched perhaps only by the brain, with its billions of neurons making the simple acts of firing off action potentials into what we perceive as the marvel of human consciousness. Similarly, each neuron and immunological cell may be quite simple in isolation, yet a part of something greater, the sum of which we don't necessarily entirely understand yet.

Beginning of modern immunology

Our ignorance is not for lack of trying and effort, however. The modern, western, mechanistic understanding of the immune system's inner workings really began at the end of the 19th century with the works of Paul Ehrlich, with his work on humoral immunity, and Ilya Mechnikov, with his work on phagocytosis, for which they both received a joint Nobel prize in Physiology and Medicine in 1908. The discoveries that came during the next 115 years spanned the description of many new cell types, from the original phagocytic neutrophils – the most prevalent immunocytes – through macrophages, monocytes, dendritic cells, baso- and eosinophils, mast cells, natural killer cells, T lymphocytes, B lymphocytes, to the more recently discovered populations of invariant natural killer T cells, innate lymphoid cells, and immune functions of cells previously thought to be only innocent bystanders, such as fibroblasts, epithelia, or indeed stromal cells..

Immune diseases advance understanding of immunology

Despite the tremendous boon to science that model organisms like mice (or chickens, in the early days) have been and continue to be to this day, a lot of our understanding of the human immune system is derived from human diseases. For instance, the first hypotheses of the role of the thymus as an organ central to the development of cellular immunity really arose during the research of Cooper, Milner and DiGeorge et al in the 1950s and 60s, which built on the cases of athymic infants, in whom these pioneers observed severe and serious infections and showed that these patients lack a substantial part of their cellular immunity – the T lymphocyte (A. DiGeorge 1965; Di George et al. 1967; Lischner,

Punnett, and DiGeorge 1967). Another example may be given in colonel Ogden C. Bruton, who observed infant boys develop recurrent sepsis caused by encapsulated bacteria, showed that these patients lack gamma globulins, and thus described a congenital agammaglobulinemia, one of the first recognized immunodeficiencies (Bruton 1952). As a final example, the work of Robert A. Good led to the first transplantation of bone marrow between persons who were not identical twins, thus treating a patient with what we now know to have been X-linked SCID (Good 1969), and demonstrated *in vivo* the role of bone marrow in the (re)constitution of the immune system.

New methods enable definition of more granular cell types

The march of progress during the 20th century built on millions of (wo)man-hours worth of scientific effort led to the invention of technologies used to dig so much deeper than the optical microscopes of Paul Ehrlich and Ilya Mechnikov allowed. The discovery of the structure of DNA by Watson & Crick (Watson and Crick 1953) eventually led to the definition of transcription factors (Roeder and Rutter 1969), key molecules that determine the fate of cells by modulating the genes a cell transcribes and by extension the proteins it synthesizes. The concept of transcription factors is to this day crucial for the identification of cellular subtypes which share common ontological and functional characteristics. New molecular biology techniques were simultaneously developed which allowed for mapping of cellular surface and intracellular protein expression patterns, which are the cornerstones of cellular subtype differentiation.

Indeed, the division of originally monolithic cellular populations which were thought to be homogeneous into an increasing number of subpopulations has ever been the trend within immunology. Within the innate branch of the immune system, monocytes can be M1 or M2 (Italiani and Boraschi 2014), dendritic cells take the form of myeloid (or conventional) or plasmacytoid cells (Guilliams et al. 2016), there's a difference between resident eosinophils and circulating eosinophils (Mesnil et al. 2016), it matters whether a natural killer cell has high (bright) or low (dim) expression of the neural cell adhesion molecule CD56 (Caligiuri 2008), mast cells can have just tryptase, or tryptase and chymase both (Buckley, Gallagher, and Walls 1998), and so forth.

Subtypes define function

These are not arbitrary divisions, but rather have vast implications for cell function and its role in the immune response; M1 monocytes are pro-inflammatory while M2 monocytes promote tissue-repair, conventional dendritic cells present all kinds of antigens to T cells but their plasmacytoid counterparts are highly specialized towards antiviral response, resident eosinophils help maintain tissue homeostasis, whereas circulating eosinophils tend to be pro-inflammatory, CD56^{dim} natural killer cells

are more potent cell killers where CD56^{bright} natural killer cells produce more cytokines, and while tryptase can be found in all mast cells throughout the body, chymase-rich mast cells are only found in the submucosa lining the gastrointestinal and respiratory tracts. In some cases, these distinctions may represent completely different lineages stemming from a common trunk of cellular origin (such as in the case of dendritic cells), but sometimes these subpopulations may only represent different stages of cellular development (such as in the case of natural killer cells) and sometimes we are not quite sure yet which may be the case.

To make matters even more complex, these divisions can also go several layers deeps, showing the fractal nature of biological systems – the deeper you look, the deeper the complexity goes (Figure 1). T lymphocytes may divide into the CD4 and CD8 expressing kingdoms within the thymus, but ahead of this decision, there splits off the population of T cells expressing not the much more common $\alpha\beta$ T cell receptor chains, but a more atypical $\gamma\delta$ version. These $\gamma\delta$ T cells have an unclear purpose, but seem to be dominant in the mucosa lining the gastrointestinal and respiratory tracts, and unlike $\alpha\beta$ T cells react to pathogens in a main histocompatibility complex (MHC)-independent manner (Nanno et al. 2007). The better-studied $\alpha\beta$ T cells divide into the aforementioned CD4 and CD8 realms, but then within CD4 T cells we have for years recognized T helper 1 (Th1) cells which are potent producers of interferon γ (IFN- γ) and stimulators of the monocyte-macrophage phagocytic system, T helper 2 (Th2) cells which produce interleukins (IL) 4, 5, 10 and 13, thus activating anti-helminthic eosinophil response and production of IgE antibodies by B cells, and T helper 17 (Th17) cells which produce IL-6, 17, 22 and tumour necrosis factor α (TNF- α) and so promote the chemotaxis and activation of neutrophils, eventually leading to potent anti-fungal and generally pro-inflammatory response (Kaiko et al. 2008).

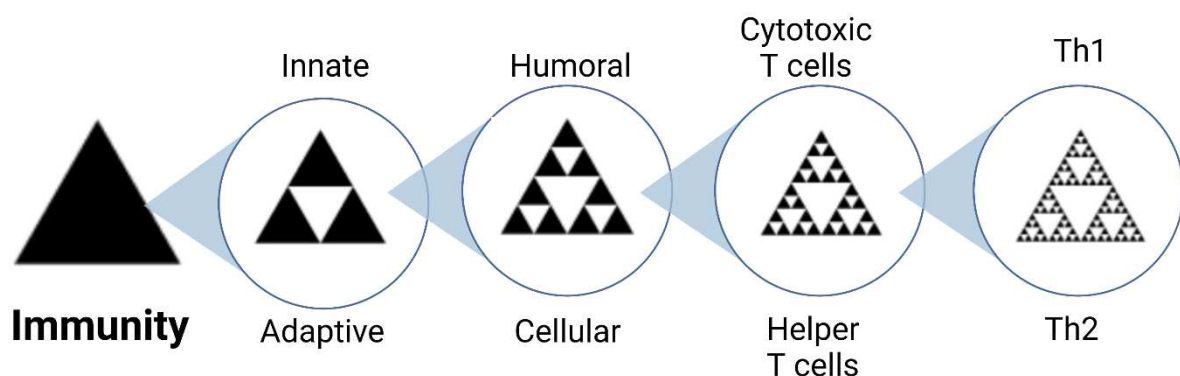


Figure 1 Fractal immunity. The deeper you look, the more you discover that previously monolithic systems are in fact comprised of different cellular lineages, lineage populations, subpopulations, clones... (This and other illustrations created in Biorender.io.)

Development of high-parametric methods

A large part of these advances in the description of immune cells, which really push the envelope of our understanding of the immune system orchestration, was the advent of flow cytometry, a technology utilizing a stream of single cells with bound antibody-fluorochrome conjugates, the excitation-emission spectra of which identify which protein is expressed to what extent by each cell. The original one-laser machines capable of measuring one or two fluorochromes (Van Dilla et al. 1969) were surpassed by two, three and now up to six laser machines, and simultaneously, the number of detectable protein targets grew from single digits in the 2000s into currently easily achievable 15+ using regular flow cytometry. The current trend of spectral flow cytometry, which does not measure individual fluorochromatic emission spectra but rather uses mathematical deconvolution methods on the whole spectrum of emissions measured at a single time to extract individual protein-antibody-fluorochrome signals, reaches over 30 detectable targets in each measured cell (Nolan 2022). Mass cytometry (cytometry by time-of-flight, CyTOF) regularly reaches over forty targets by conjugating antibodies to heavy metal ions and measuring the mass of ions emitted from a vaporized cell, which eliminates spectral overlap, at the cost of higher price per sample and higher requirement for starting material volume (Bandura et al. 2009).

While single-cell RNA sequencing (scRNA-seq) has been available for decades at this point (Eberwine et al. 1992), it has become more wide-spread with the development of next generation sequencing (Tang et al. 2009). Its great strength is the capability of assessing the transcriptomic status of a cell with regard to all 20 thousand+ transcribed genes. However, it provides no insight into actual protein expression, which may be further modulated by post-translational modifications, metabolic status of a cell and other factors. This hurdle is now overcome by cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), which uses oligonucleotide-labelled antibodies to simultaneously map an integrated single-cellular transcriptome with hundreds of protein targets per experiment (Stoeckius et al. 2017). In tissues, this approach can be extended into 2 dimensions with spatial CITE-seq, a truly cutting-edge technology (Ben-Chetrit et al. 2023). An alternative approach enables concurrent analysis of gene expression and epigenetics – chromatin accessibility – by combining simultaneous high-throughput assay for transposase-accessible chromatin (ATAC) and RNA expression with sequencing (SHARE-seq) (S. Ma et al. 2020), or chromatin accessibility and protein expression by ATAC with select antigen profiling by sequencing (ASAP-seq) (Mimitou et al. 2021). These methods can then be combined together to create multi-modal atlases of cells spanning transcriptomics, epigenetics as well as proteomics at the same time (Hao et al. 2021).

On modern analysis and AIs

All of these methods create huge datasets, which would be exceedingly difficult – if not impossible – for humans to interpret into insightful biological hypotheses or thought constructs without external assistance. Going through thousands of cells and analysing the expression levels of 20 thousand genes in each of them would be massively time-consuming and we would by nature default to comparing the few already known populations against each other for one gene (or in the case of CITE-seq or high-parametric flow cytometry, one protein) at a time. With this approach, however, we would wilfully ignore the amazing complexity of the biological system analysed, with its patterns of co-expressing genes and proteins, analogue/non-binary levels of positivity for markers, spatial relationships between cells, and so forth. For this reason, the advent of these high-parametric approaches necessitated also the development of computational semi- or unsupervised approaches to extract information which we CAN interpret, test biologically and potentially implement in disease diagnosis or treatment (Fuda et al. 2023; Hu, Bhattacharya, and Butte 2022).

In late 2022, the world was irrevocably changed by the unveiling of ChatGPT (Generative Pre-trained Transformer), a generative artificial intelligence language model which was freely available to everyone, did not require technological knowledge or specialized software to use, and as a result spread like wildfire, with over 1 million users after only 5 days, 100 million users after two months and over 1.5 billion site visits after 8 months. Such success is unprecedented in modern day (or indeed any) technology and heralds a shift of artificial intelligence topics from obscurity and the world of scientific fiction literature to the forefront of public discourse. While the specific technological background as well as the expected impact of these new technologies exceeds the scope of both this work and the expertise of its author, it is clear that neural networks and machine learning models which utilize them are here to stay and will transform the way we do research.

Nevertheless, to assess the potential benefit of AI for science and immunology in particular, I believe it is necessary to have some limited understanding of how a computer system can write jokes, recipes or obituaries, or – perhaps more helpfully for the average scientist – summarize any text into a 250-word abstract or prepare quiz questions for students. Thus, a brief look into the structure of ChatGPT is warranted.

In its essence, the GPT-3 is based on a 96-layer neural network with 2048 input slots, each of which can take one of 50 257 “tokens” (usually those are words of the English language). However, tokens (or words) don’t stand alone, a “dog” is not just “token number 249”, but rather a series of 12 288 dimensional coordinates which define how the word “dog” usually behaves in written language in relation to each of the other 50 256 words in the sum of text the system “pre-trained” on. In the case

of GPT-3, this sum of text spans about 45 terabytes (!) of data, the exact composition of which is proprietary and thus secret. Suffice to say, however, that its impressive 500 billion words, if printed as books and lain on top of each other, would form a stack over 160km tall. Whatever input the system is provided is then processed by nodes (or “neurons”) in 96 layers, which interconnect between each other with various strengths, both stimulatory or inhibitory (“weights” and “biases”), altogether defined by 175 billion (!) parameters. The result of this vast sum of data fed into the neural network with all of its parameters is a unique combination of output words, which best correspond to the initial query. Clearly, the system is not omniscient and does not really understand anything it produces, but it has a vast array of information to derive its responses from. With this, however, arrive some challenges, which are especially pertinent for its potential use in science – if the training dataset/corpus does not contain some information, the system cannot intuit it, and worse still, it does not logically evaluate the validity of its claims, if they fit the dataset it was trained on. On the other hand, it’s great at contextualizing and connecting disparate pieces of information on a similar topic. This is something all scientists utilizing ChatGPT or other similar networks must be aware of.

With all its fallibility, however, the structure of GPT and similar neuronal networks mimics that of biological brains. In those, layers of neurons interconnect with axons, which can secrete small or large amounts of stimulatory or inhibitory neurotransmitters and the unique connections between them are what determine our reflexes and even our personalities. I once again posit that it also mimics the structure of the immune system – each cell senses the input of hundreds of surface and cytoplasmic receptors and based on its inherent “weights and biases” (e.g. presence of proteins and transcriptional factors, openness of chromatin, expression of unique cellular receptors, etc.) produces responses – changes in cell cycle, production of a plethora of cytokines, changes in gene and protein expression, etc. The cell then, in turn, interacts with other immune cells, which may be structured in 3D in a secondary lymphatic organ such as a lymph node, which have their own weights and biases and the complex sum of interactions between these cells results in a very unique form of immune responses (Figure 2).

The complexity of these systems results in some incredible outcomes – be it consciousness, the human-like responses of ChatGPT, or the coordinated defence of the body against a virus. Nevertheless, it also means that we don’t always quite understand why the complex system does what it does – the so-called “black box” problem. We understand the inputs, we understand the outputs (most of the time), but not exactly why the system reached the conclusion it did. It is my belief, that the advances in the theory of mind, in computing and in artificial intelligence will be extremely helpful in unravelling the inner workings of the immune system as well.

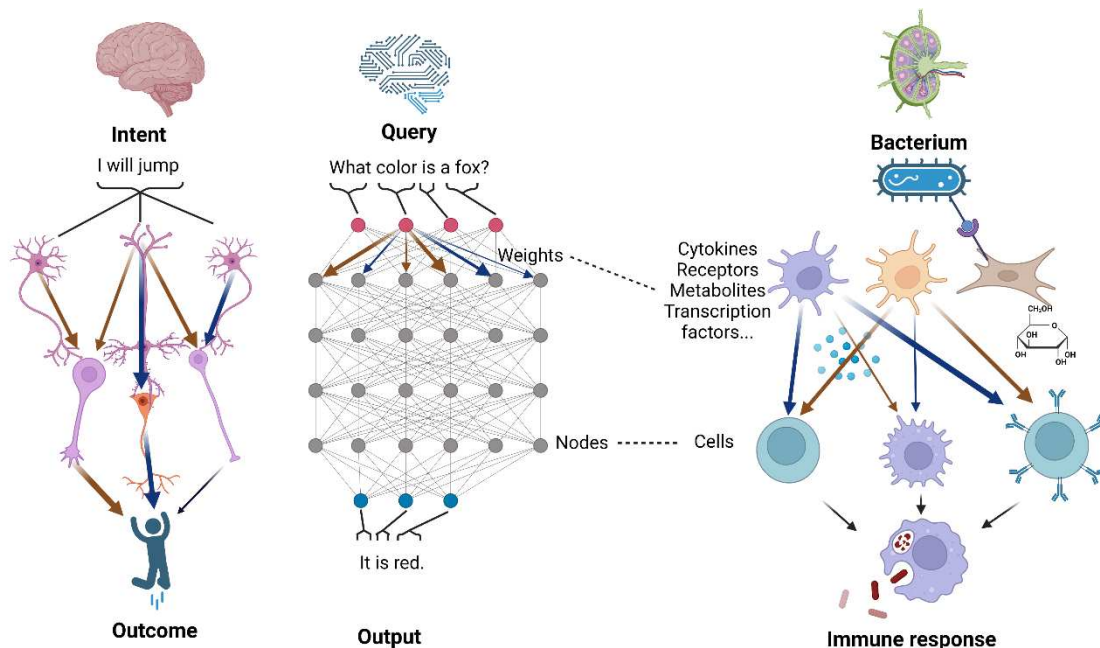


Figure 2 Immune brain. Left, neuronal network within the human brain, its interconnecting neurons with stimulatory and inhibitory synapses. Center, deep learning artificial neuronal network, its interconnecting nodes with positive and negative weights. Right, immune brain, its cellular components with stimulatory and inhibitory cytokines, transcription factors, specific receptors etc. Upon the input of data, each system processes the input through many layers of interconnected neurons/nodes/cells and in the end, an outcome arises – be it jumping up, informing the user that a fox is red, or phagocytosing a bacterium.

The use of computer-assisted data analysis in immunology

Indeed, computers have been assisting us with the complexities of immunological research for quite some time. For many years now we have been using principal component analysis (PCA) to reduce the expression of several cellular surface/cytoplasmic/nuclear markers into (usually) two dimensions, which we have evolved to understand better than 4+ dimensional data. Nowadays, more methods like Uniform Manifold Approximation and Projection (UMAP) (McInnes, Healy, and Melville 2018), t-Distributed Stochastic Neighbour Embedding (tSNE) (Gmail and Hinton 2008), fast Fourier transform (FFT)-accelerated interpolation-based t-SNE (fit-SNE) (Linderman et al. 2019), vi-SNE (Amir et al. 2013) which all achieve a similar end result have gained a lot of popularity, each developed specifically to best fit a specific type of dataset (e.g., mass cytometry data, scRNA-seq, etc).

Reducing several dimensions into two may be helpful for visual identification of similar cells, however, the best no-loss approach is to use non-reduced data. For such identification of similar cellular clusters, variations of nearest-neighbour algorithms are commonly used (Hu, Bhattacharya, and Butte 2022), such as k-nearest neighbour (KNN) (Cover and Hart 1967), flow cytometry self-organizing maps

(FlowSOM) (Van Gassen et al. 2015), EmbedSOM (Kratochvíl, Koladiya, and Vondrášek 2020), PhenoGraph (Levine et al. 2015), and more.

These can then be combined with neural networks to improve the quality of dimensionality reduction (Espadoto, Hirata, and Telea 2021). Neural networks can also be used to combine different datasets measured across experiments, a way to circumvent the limitations outlined above for combination of transcriptomics, epigenetics, proteomics. For example, Lin et al have used neural networks to combine annotated data from scRNA-seq, scATAC-seq, as well as multimodal data from CITE-seq and ASAP-seq experiments, with comparable or better accuracy than other tools (Lin et al. 2022). Alternatively, machine learning can extract developmental trajectories of cells (Maslova et al. 2020)

Neural networks and artificial intelligence are now being used not only to process raw data on cellular populations but are being leveraged to tackle any large datasets. Crucially, all of these methods rely on datasets with not only many parameters, but more importantly, many individual entries (be those cells or patients). For this reason, the global pandemic of COVID-19 saw a boom of neural network/machine-learning analyses of large patient cohorts, which were used to predict outcome and perform risk-stratification of patients – already in September 2021, a meta-analysis by Wang et al analysed 78 studies discussing AI-assisted diagnosis for COVID-19, as did many more (Martinez et al. 2022; L. Wang et al. 2021; Mann et al. 2022; Chang et al. 2021).

Now that the pandemic is over, other huge cohorts are being leveraged to feed data into the black boxes of neural networks, finding predicting factors of cancer immunotherapy outcomes (Yang et al. 2022; Ghaffari Laleh et al. 2023), immune-related processes underpinning different courses of the disease (C. Zhang et al. 2022), and of course trawling medical images for cancer-specific patterns – in skin cancer (Tschandl et al. 2020), gastric cancer (Hirasawa et al. 2018) or others (Bi et al. 2019).

Similar approaches are used in the field of autoimmune diseases. AI has been used to predict diseases risk and identify novel risk factors in inflammatory bowel diseases, type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, diagnose clinically unclear cases, monitor disease progression and outcome in psoriasis, RA, IBD and coeliac disease, and monitor the quality of disease management (Stafford et al. 2020). Finally, AI can be used to identify patients in need of autoimmune disease testing using electronic health records (Forrest et al. 2023).

In the field of primary immunodeficiencies, such approaches are limited by the relative scarcity of patients – primary immunodeficiencies are rare diseases which may be fairly common in bulk, but each disease may only be represented by a handful of patients in each country and have a completely unique pattern of laboratory findings or symptoms, which limits the possibility of bulk analysis. For example,

there's currently 8 patients with activated phosphoinositide-3-kinase delta syndrome (APDS) in the Czech republic (Marketa Bloomfield et al. 2021) **(Addendum II)**, about 10 patients with chronic mucocutaneous candidiasis caused by Signal Transducer And Activator Of Transcription 1 (STAT1) gain-of-function mutations (Marketa Bloomfield et al. 2022), 1 patient with X-linked inhibitor of apoptosis protein (XIAP) deficiency (Parackova, Milota, et al. 2020), 3 patients so far with congenital athymia treated with a thymic transplant (Klocperk and Šedivá 2019) **(Addendum II)**, and even the most prevalent symptomatic primary immunodeficiency, common variable immunodeficiency (CVID), features only just over 330 patients in the whole country – and in its case, huge disease heterogeneity further splits the dataset.

Since diagnosing patients with rare diseases among the vast majority of patients with more common illnesses can feel like searching for a needle in a haystack, one American team has now successfully validated an AI-based pipeline for population-wide primary immunodeficiency screening (Rider et al. 2023; Mayampurath et al. 2022), utilizing electronic health records to identify patients suitable for a referral to a specialist.

Still, even once patients are referred to a centre, the huge number of recognized primary immunodeficiencies (which now exceeds 480 unique entities) makes it impossible even for specialized clinicians to always bear all of them in mind and direct diagnostic procedures in the most probable direction in an unbiased fashion. For this reason, I teamed up with data science and programmer colleagues to develop a web-based app to help with diagnosis of primary immunodeficiencies – PIDLA (Primary ImmunoDeficiency Look-up App). This tool utilizes the IUIS primary immunodeficiency classification paper (Tangye et al. 2022) and the Human Phenotype Ontology (HPO) database and allows clinicians to look up the unique combination of clinical and laboratory features of their patients, searching for the most likely diagnosis based on their unique combination of symptoms/features (Klocperk 2022) **(Addendum II)**. While the tool doesn't use neural networks, it utilizes complex mathematics in a 350-dimensional space to instantly deliver the most fitting diagnosis, which would've been impossible without the aid of modern computing.

THE IMPORTANCE OF MAINTAINING A DYNAMIC EQUILIBRIUM BETWEEN HUNDREDS OF CELLS AND CYTOKINES: IMMUNODEFICIENCIES, AUTOIMMUNITIES AND EVERYTHING IN BETWEEN

With these advances in both our understanding of the intricate populations of cells and the way they interact with each other, it has become obvious that the proper immune homeostasis of the immune system is an ever-adjusting, delicate and dynamic equilibrium of interactions between thousands of cells located in different spacetimes, with different metabolic and activation statuses, communicating via different cytokines, with different receptors, etc.

One of the most important shifts in our understanding of the (human) immune system was ridding ourselves of the black-and-white division between immunodeficiency (e.g. when the immune system is too weak and this results in a propensity towards infections) and autoimmunity (e.g. when the immune system is too active and this results in damage against host tissues). We now recognize a growing number of immune dysregulation diseases (Tangye et al. 2022) where the particular way, in which the immune system is compromised, leads to both of the above.

APECED as a demonstration of immunodeficiency with autoimmunity

A classic example may be autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy (APECED) syndrome, a disease classified as primary immunodeficiency due to one of its defining symptoms, chronic mucocutaneous candidiasis, which nevertheless also presents with early onset type 1 diabetes (e.g. autoimmune response against the insulin-producing beta cells in the pancreas), autoimmune thyroiditis, autoimmune hepatitis or adrenal insufficiency (e.g. autoimmune response against the adrenal glands) (Ferré, Schmitt, and Lionakis 2021). The core of this multifaceted diseases lies in its pathophysiology, which is caused by a faulty transcription factor Aire, resulting from autosomal recessive loss-of-function mutations of its coding gene, *AIRE*. This transcription factor is a key mediator of negative selection of developing T cells in the thymus. As central coordinators of the immune response, much evolutionary pressure is exerted on T cells to not react (and thus initiate responses) against host tissues and thus Aire induces expression of tissue-specific antigens in the thymus, inducing tolerance of developing T cells against these antigens. When this process fails, such as when Aire is dysfunctional, autoreactive T cells develop and with them so do the clinical autoimmune diseases.

Candidiasis may seem like an unlikely component of this disease, as it is an infection and as such should result from faulty defence against the fungal pathogen, rather than an overactive immune system. Its cause may be surprising, until we realize that components of the immune system are also, in essence,

host tissues. In patients with APECED, the breakdown of negative selection leads to development of autoimmune response against not only beta cells or the thyroid, but against components of the immune system also. Patients with APECED create autoantibodies against IL-17 and IL-22, key cytokine factors in the mobilization of neutrophils and resolution of fungal infections (Kisand et al. 2010)(Figure 3). As a side-note, a recent study of a friendly research group revealed that extra-thymic expression of Aire in MHC-II-expressing type 3 innate lymphoid cells (ILC3) also contributes to development of functional response against *Candida* infection by promoting activation and proliferation of *Candida*-specific Th17 cells, revealing a new mechanism which may contribute to the pathogenesis of this interesting diseases (Dobeš et al. 2022).

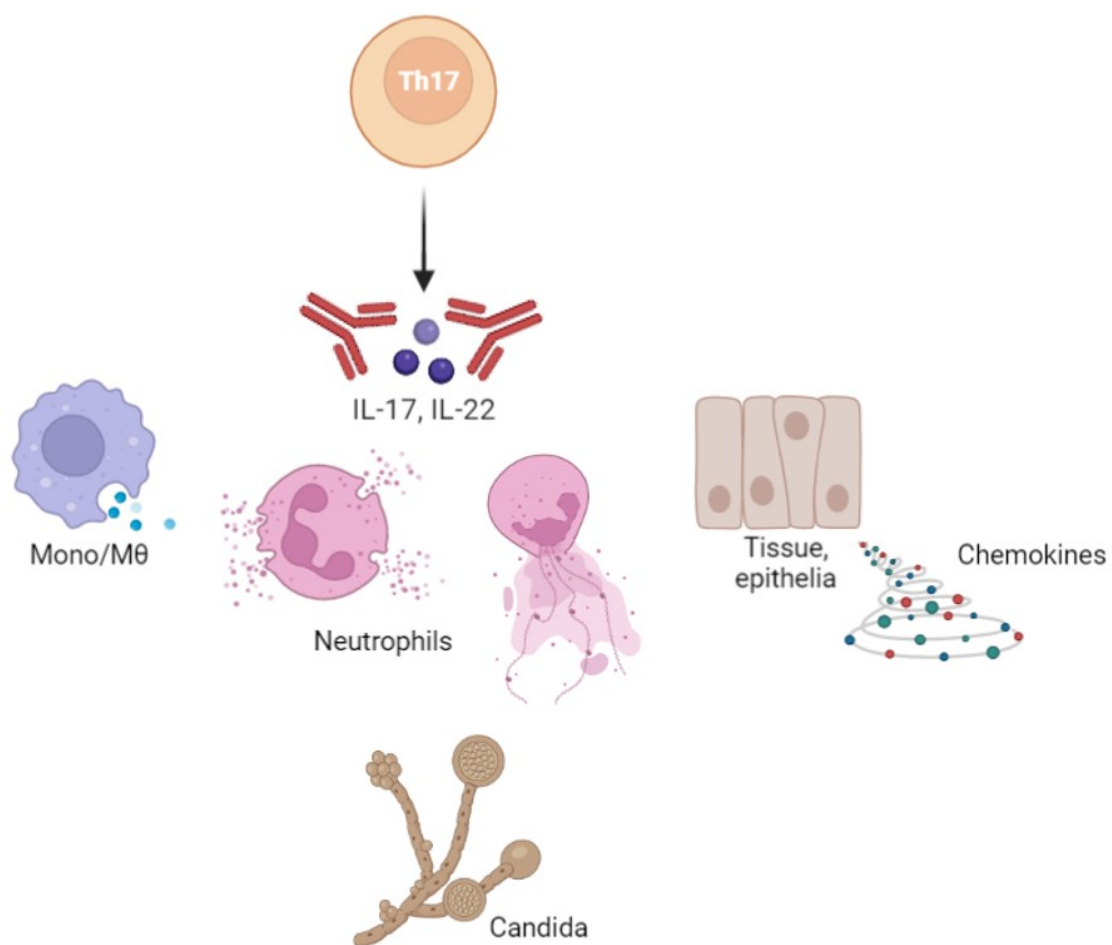


Figure 3 Role of Th17 cells and anti-IL17/anti-IL22 antibodies in defence against fungal infections. Th17 cells produce IL-17 and IL-22 cytokines which promote monocyte/macrophage phagocytosis, neutrophil degranulation and NETosis and promote pro-inflammatory state in the epithelia, which positively feeds back into immunocyte chemotaxis. Blockade of IL-17 and IL-22 activity by autoreactive antibodies thus results in impaired defence against fungal pathogens.

Anti-cytokine antibodies as a risk factor for severe COVID-19

When the COVID-19 pandemic spread around the globe, we joined forces with the COVID Human Genetic Effort (HGE) initiative lead by Prof. Jean-Laurent Casanova from the Rockefeller University and Prof. Helen Su of the National Institute of Health. The goal of this international initiative was to understand the genetic and immune factors which lead to severe course of COVID-19 in some individuals, and which may be used to select high-risk individuals and through higher vigilance provide them with better health outcomes.

Together and fittingly to the concept of immune (dys)regulation outlined at the start of this chapter, this initiative identified the presence of anti-type I interferon antibodies to be a strong determinant of severe course of COVID (Q. Zhang et al. 2020; Asano et al. 2021). Type I interferons are particularly important in anti-viral defences – they are primarily produced by plasmacytoid dendritic cells and induce an antiviral state in surrounding tissues, but can also potentiate cytotoxicity in CD8 T cells and NK cells. When blocked by anti-cytokine antibodies, patients respond less efficiently against the virus, which leads to higher replication rates and overall increases the morbidity and mortality. Although these antibodies are particularly detrimental for overall outcome in young adults, who have otherwise good prognosis, their prevalence increases dramatically with age, especially in people over 60 years of age (Manry et al. 2022; Bastard et al. 2021). Notably, genetic mutations in antiviral defence factors – such as toll-like receptor 7 (TLR7), which senses single-stranded RNA, or those linked to type I interferons, such as interferon response factor 7 (IRF7) – were also associated with severe course of the disease (Q. Zhang et al. 2020; Asano et al. 2021).

Locally, we worked with colleagues in the intensive care and COVID units and showed that patients with severe course display hyperactivation of cytotoxic CD8 T cells and suffer from hepato-biliary lesions, compared to outpatients and patients with milder form of the disease (Klopperk, Bloomfield, et al. 2020). In this work we also utilized some systemic biology approaches to demonstrate that in patients with mild course of COVID, markers of inflammation correlate with immunoglobulin levels and antiviral antibodies, as one would expect during a functional immune response, whereas in those with severe course, markers of inflammation correlated instead with elevated liver and biliary enzymes and markers of tissue damage, showing the inflammation turning self-destructive. In a separate study we analysed components of the innate immunity, which are the first responders to infection. Neutrophilia is one of the most strongly associated laboratory features of severe COVID (D. Wang et al. 2020), and we were able to show that the neutrophils of COVID patients were primed to induce a pro-inflammatory Th17 response in T cells (Parackova et al. 2021). They had enhanced degranulation of myeloperoxidase and neutrophil elastase, produced more inflammatory cytokines IL-1 β , IL-6 and

TNF- α and had higher basal levels of interferon signature genes *IFNA*, *IFIT1*, and *ISG15* (Parackova, Zentsova, et al. 2020). These studies highlight the importance of balanced immune responses, as too strong a response can do as much harm to the host organism as one too weak (Figure 4). This concept will be explored further in the section on T cell exhaustion.

Too much of a good thing – MIS-C, Hck, Caspase 8

Another illustration of the necessity for a balanced immune response is the multisystem inflammatory syndrome in children (MIS-C), which is one of the most important sequelae of COVID-19 in paediatric patients. While children very rarely suffer from severe course of the actual infection, in a small subset, a potent multisystemic inflammatory response is triggered some 4-6 weeks after the infection. Clinically it is somewhat heterogeneous, but usually very closely resembles Kawasaki disease, another paediatric inflammatory disease with unknown aetiology (Sancho-Shimizu et al. 2021). We were among the first to document and publish the course of one such patient (Klocperk, Parackova, et al. 2020)(**Addendum II**), who was successfully treated with systemic steroids and high-dose immunoglobulins, an approach which has later become the main cornerstone of MIS-C management world-wide. Throughout the rest of the pandemic period, we coordinated a group of paediatric immunologists and intensive care specialists in contributing the Czech MIS-C patients into the COVID-HGE consortium, which later resulted in a publication showing that ~ 1% of MIS-C cases may be underpinned by autosomal recessive deficiencies in the OAS1, OAS2, RNase L pathway. This intracellular pathway is formed by type I interferon-inducible double-stranded RNA-sensing proteins OAS1 and OAS2 which generate 2'-5'-linked oligoadenylates, thus activating the antiviral single-stranded RNA-degrading RNase L. A breakdown of this viral nucleic acid-degradation system results in cellular hyperstimulation and continuous production of proinflammatory cytokines, thus potentially triggering the multisystem inflammation underlying MIS-C.

We have then used the Czech cohort to show that MIS-C patients hyperproduce IFN- γ , but more uniquely, that B cells are also significantly affected in MIS-C patients compared to healthy post-COVID children. In particular, MIS-C patients have vastly increased serum levels of B cell survival cytokines BAFF and APRIL, yet lose circulating plasmablasts, suggesting a breakdown of humoral immune response coordination in response to the infection (Klocperk et al. 2023). This theory of B cells response dysregulation is further supported by the presence of anti-nuclear antigen autoantibodies present in MIS-C patients but not healthy post-COVID children – indeed other studies have shown an increase of double-negative IgD⁺CD27⁻ activated B cells in MIS-C (Ramaswamy et al. 2021) and others have shown the same in SLE where the production of autoantibodies is an integral part of disease pathology (Tanaka et al. 2018).

This theorem of “too much of a good thing” has also underlain an exciting description of a novel primary immunodeficiency we’ve been proud to contribute to. Our sister group at the Department of Paediatric Haematology, Motol University Hospital, has identified a gain-of-function mutation in the Hematopoietic cell kinase (Hck) in a patient with early onset pulmonary and cutaneous vasculitis attended to at the Department of Paediatrics (Kanderova et al. 2021). This mutation led to enhanced production of ROS, proinflammatory cytokines and increased phosphorylation in transfected granulocytic HL60 cell lines, as well as more mature surface phenotype both in native state and after stimulation with bacterial ligands. These changes caused cutaneous and pulmonary bleeding, which lead to continuous reliance on oxygen and repeated bouts as well as long-term immunosuppression, which in turn increased susceptibility to infections, triggering more inflammation etc. We were able to repurpose the tyrosine kinase inhibitors ruxolitinib and dasatinib used in similar conditions with increased interferon signatures and vasculopathy such as STING-associated vasculopathy with onset in infancy (SAVI) to partly abrogate the molecular changes and transiently improve patient’s phenotype, or at least slow down her deterioration.

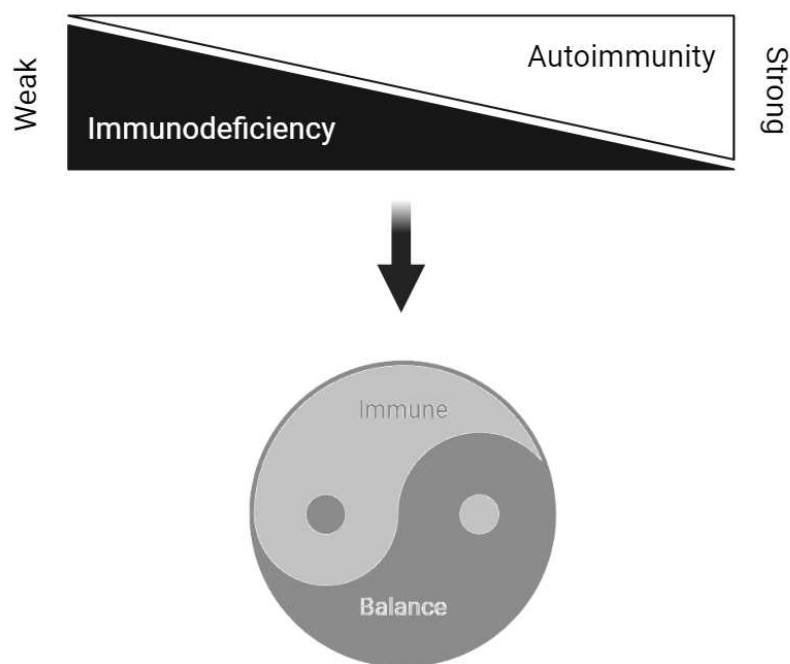


Figure 4 Yin-yang of immune balance. We have moved past the black vs. white of immunodeficiency vs. autoimmunity and embraced the different shades of grey and white-in-black of immune balance and regulation.

DYSFUNCTIONAL T CELL DEVELOPMENT UNDERLIES MANY RARE PRIMARY INBORN ERRORS OF THE IMMUNITY

As outlined above, both an overly strong and mistargeted immune response may result in the clinical manifestation of infection, which may counter-intuitively go hand in hand with autoimmunity. This paradox of modern immunology is well illustrated in a number of rare inborn errors of the immunity treated at the Motol University Hospital.

The Th1:Th17 balance, STAT1 and STAT3

Frequently, mutations in the master coordinators of immunity, the T cells, underlie changes that cascade throughout the rest of the immune system. In some cases, these locate into signal transducer and activator of transcription (STAT) genes. STATs are a family of transcription factors that are recruited by cytokine receptor-associated Janus protein kinases JAK1 and JAK2, upon phosphorylation dimerize, bind to DNA and induce differentiation of naïve CD4 T cells into subsets with distinct functional phenotypes, such as Th1 and Th17 (Maródi et al. 2012). While recent advances in the methodology outlined above and our better understanding of the vast diversity of individual cellular functional profiles steer us away from the canonical dissection of T cells into two or three subsets each characterized by the production of a single cytokine (e.g. Th1 cells producing IFN- γ , Th2 cells producing IL-4, Th17 cells producing IL-17, etc.), this simplification has been helpful in explaining the pathophysiology of diseases associated with STAT mutations.

STAT1 gain-of-function

For example, gain-of-function mutations in STAT1 lead to preferential differentiation of T cells into the IFN- γ -producing Th1 phenotype and competitive inhibition of T cell differentiation down the STAT3-dependant IL-17-producing Th17 route. Since Th17 cells are vital for the recruitment of neutrophils and mobilization of anti-fungal defence, patients with STAT1 gain-of-function mutations characteristically suffer from chronic mucocutaneous candidiasis. We have shown that blocking the upstream JAK proteins with the selective inhibitor ruxolitinib results in lower phosphorylation of STAT1 after IFN- γ stimulation and amelioration of clinical candidiasis, in a reversible manner (Markéta Bloomfield et al. 2018). More in-depth description of STAT1 gain-of-function associated phenotypes belong in the hands of local specialists, especially Dr. Bloomfield and Dr. Parackova, however, I find it fascinating to point out that patients with STAT1 gain-of-function mutations suffer not only from an abundance of fungal infections (for reasons outline in the paragraph above), but also develop striking non-infectious complications, such as different autoimmune disorders (autoimmune hypo/hyperthyroidism, type 1 diabetes or inflammatory bowel disease) and development of

aneurysms (Toubiana et al. 2016). Cells of patients with STAT1 gain-of-function mutations also respond hyperactively to type I interferons and overexpress genes associated with interferon signature (Kaleviste et al. 2019), thus overlapping substantially with the clinical phenotype of autoinflammatory type I interferonopathies and SLE. This fascinating disorder well illustrates the breadth of clinical and laboratory features caused by cascading biological processes resulting from a potentially simple single genetic change, especially when it's prominent in the central coordinators of the immune response, the T cells.

STAT3 loss-of-function

Interestingly, loss-of-function defects in STAT3, which is another STAT transcription factor co-responsible for the differentiation of T cells into IL-17-producing Th17, result in a different phenotype which far exceeds the propensity towards mucocutaneous candidiasis seen in STAT1 gain-of-function patients and doesn't share its other associated clinical complications. These loss-of-function mutations in STAT3 are inherited in an autosomal dominant manner and in addition to lack of Th17 cells are characterized by extremely high serum levels of immunoglobulin E, giving the disease its name "autosomal dominant hyper-IgE syndrome", or "AD-HIES" for short.

When we reviewed the features of AD-HIES patients we noted that in addition to fungal infections these include atopic disposition with frequent development of allergic diseases, eczema, characteristic craniofacial dysmorphism, skeletal and dental anomalies, frequent soft-tissue infections with characteristic "cold" abscesses caused by *Staphylococci* ("cold" due to lack of Th17-induced infiltration by neutrophils and local hyperaemia) and other features which may not be explained by Th1:Th17 dysbalance (Ponsford, Rae, and Klocperk 2018). The lack of STAT3 transduction abrogates signalling downstream of several cytokine receptors, including the proinflammatory IL-6 (contributing to the lack of Th17 cells) but also including the anti-inflammatory IL-10 (which leads to persistent hyperinflammation especially in the lungs, resulting in the formation of pneumatocoeles characteristic for the disease (Freeman and Holland 2009)).

The increased production of IgE is not a completely explained feature of the disease and may not be explained by missing Th17 cells. The role of IL-6 in the homeostasis of IgE has been proposed (Spencer et al. 2019) and may contribute, as might the STAT3-independent activity of IL-4 (Pène et al. 1988).

Notably, HIES can also be caused by other genes active in the IL-6-STAT3 signalling cascade, such as mutations in the gene coding the IL-6 receptor, IL-6 signal transducer protein, or ZNF341 transcription factor which positively regulates the expression of STAT3, as we reviewed elsewhere (Ponsford et al. 2018).

STAT3 gain-of-function

The ubiquitous activity of STAT3 which leads to syndromic skeletal and other anomalies in AD-HIES is well illustrated on STAT3 gain-of-function patients, in whom immunodeficiency is accompanied by lymphoproliferation (such as generalized lymphadenopathy or splenomegaly), autoimmune cytopenias (Coombs-positive anaemias, autoimmune thrombocytopenias and neutropenias), interstitial lung disease with infiltration of lymphocytes into the pulmonary interstitium and in over half of patients, growth delay (Leiding et al. 2022). Interestingly, majority of patients also suffer from eczema, which is also a common finding in STAT3 loss-of-function AD-HIES (as in bearing an “opposite” mutation).

Severe growth failure and impairment of the growth-hormone axis was a striking finding in our local patients. Since growth hormone signals through STAT5b, which is modulated by STAT3 (Kofoed et al. 2003), we explored the signalling through STAT5 in patient cells and found it impaired (H. Sediva et al. 2017), possibly explaining the growth failure seen in STAT3 gain-of-function patients. It is curious, that the craniofacial dysmorphism observed in STAT3 loss-of-function appears to be – at least anecdotally in two patients assessed at the Department of Genetics, Motol University Hospital, an inversion of the one seen in STAT3 gain-of-function (Figure 5).

We further contributed to the work of our Swiss colleagues, whose patient had very severe transfusion-dependant anaemia. In their work they’ve shown fewer erythroid precursor cells and impaired response to erythropoietin (EPO), which requires STAT3-suppressed STAT5 signalling downstream of the EPO receptor to properly exert its biological effect and promote erythropoiesis (Mauracher et al. 2020). They’ve been able to show that this impairment can be ameliorated by combined immunosuppression through cyclosporin and tocilizumab, restoring the patient’s ability to produce sufficient red blood cell counts.

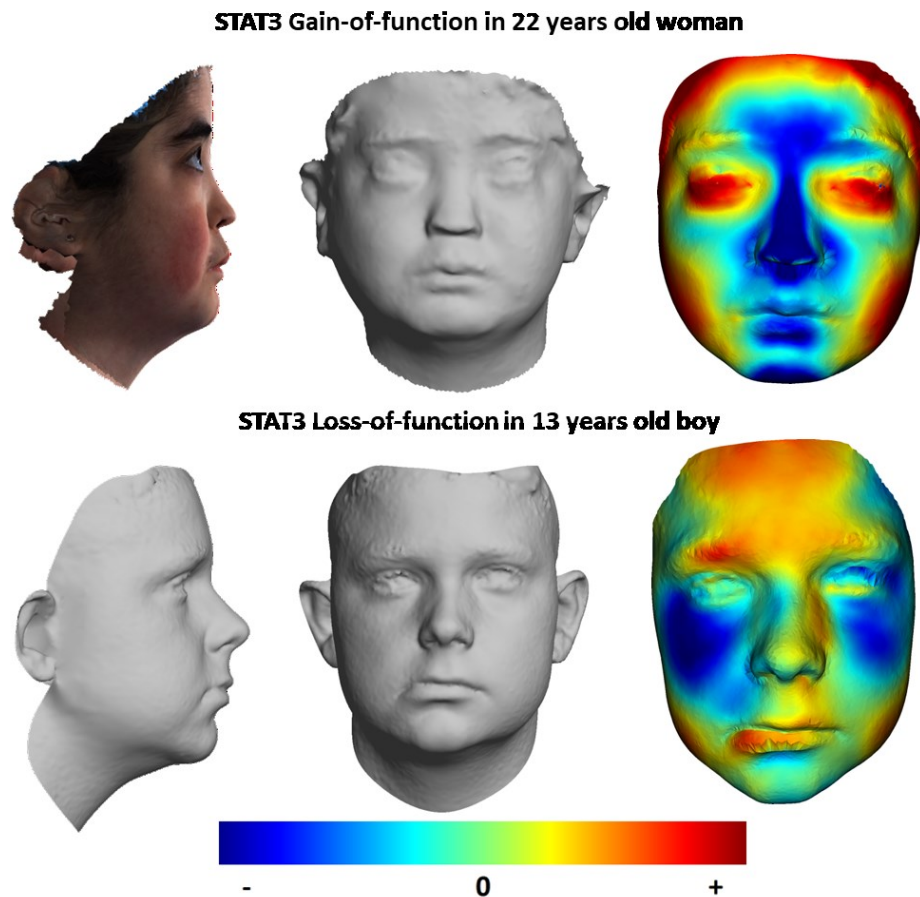


Figure 5 Craniofacial dysmorphia in STAT3 gain/loss-of-function. An interesting inversion of facial 3D structure mapped in one STAT3 gain-of-function and one STAT3 loss-of-function patient. Credit to Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University and Motol University Hospital.

THE BIRTHPLACE OF T CELLS, THE MASTER IMMUNE COORDINATORS – THE THYMUS

The inborn errors of the immunity outlined in the section above share a common feature, a dysfunctional T cell. T cells behave as both central regulators of the immune response and its effectors and have been the focus of immunological investigations since the beginning of clinical immunology. DiGeorge syndrome named after Professor Angelo DiGeorge was one of the first described immunodeficiencies and has been the focus of our continued study at the Department of Immunology at the Motol University Hospital. A more exhaustive elaboration on the pathogenesis and clinical features of DiGeorge syndrome can be found in my dissertation (Klocperk 2019), however, a more concise summary will be helpful for contextualization of our combined results that will follow.

In brief, DiGeorge syndrome was originally described as a combination of congenital absence of the thymus and impaired cellular immunity (A. DiGeorge 1965). Patients with the originally described DiGeorge syndrome chiefly presented with hypoparathyroidism (and thus hypocalcaemic seizures), lack of thymus, recurrent infections (including *Monilinia* oral thrush) yet at the same time a failure to develop hypersensitivity following *Monilinia* provocation, and failure to reject skin grafts. With this, DiGeorge supported the theory of Cooper, Peterson and Good, who in chickens demonstrated that thymus and bursa Fabricii are sources of two distinct lymphocyte lineages, which we now of course recognize as T and B cells respectively (Cooper, Peterson, and Good 1965). Later, they together described a novel human disease that later came to be known as DiGeorge syndrome (A. M. DiGeorge et al. 1967).

The molecular cause of DiGeorge syndrome was first elucidated in 1992 by Driscoll et al., as deletions on the long arm of chromosome 22 in the 22q11.2 region (D. a. Driscoll, Budarf, and Emanuel 1992). These deletions, however, have also been shown by the same group in “velo-cardio-facial syndrome”, characterized by autosomal dominantly-inherited disorder of palatal anomalies, cardiac defects, learning disabilities and typical craniofacial dysmorphia (C. S. Ma et al. 2012), pointing at substantial clinical variability. There is thus a great variability in the clinical penetrance of the 22q11.2 deletion, which has a population prevalence between 1:4,500-1:10,000 - only about two thirds of patients bearing it present with cardiac anomalies (most frequently of the outflow tract, characteristically the tetralogy of Fallot – pulmonary vein stenosis, ventricular septal defect, overriding aorta and right ventricular hypertrophy), only two thirds have velo-palatal defects, etc (Sullivan 2019). Further confusion is added by other possible causes of the same clinical phenotype, including deletions on the 10th chromosome (sometimes called DiGeorge syndrome 2) (Pignata et al. 1996), monogenic mutations of genes in the 22q11.2 region, such as TBX1 (Yagi et al. 2003), or the role of antenatal exposure to

teratogens, such as retinoic acid or hyperglycemia (Coberly, Lammer, and Alashari 1996; Roberts et al. 2005; Gosseye et al. 1982).

Due to the variable phenotype associated with identical genetic deletions, the association of symptoms and genetic findings was for a time called CATCH22 (cardiac defects, abnormal facial features, thymic hypoplasia, cleft palate, hypocalcaemia, chromosome 22 deletions)(Figure 6), which was well-descriptive, but for obvious literary connotations and associations with a “no win” situation fell out of use (Burn 1999).

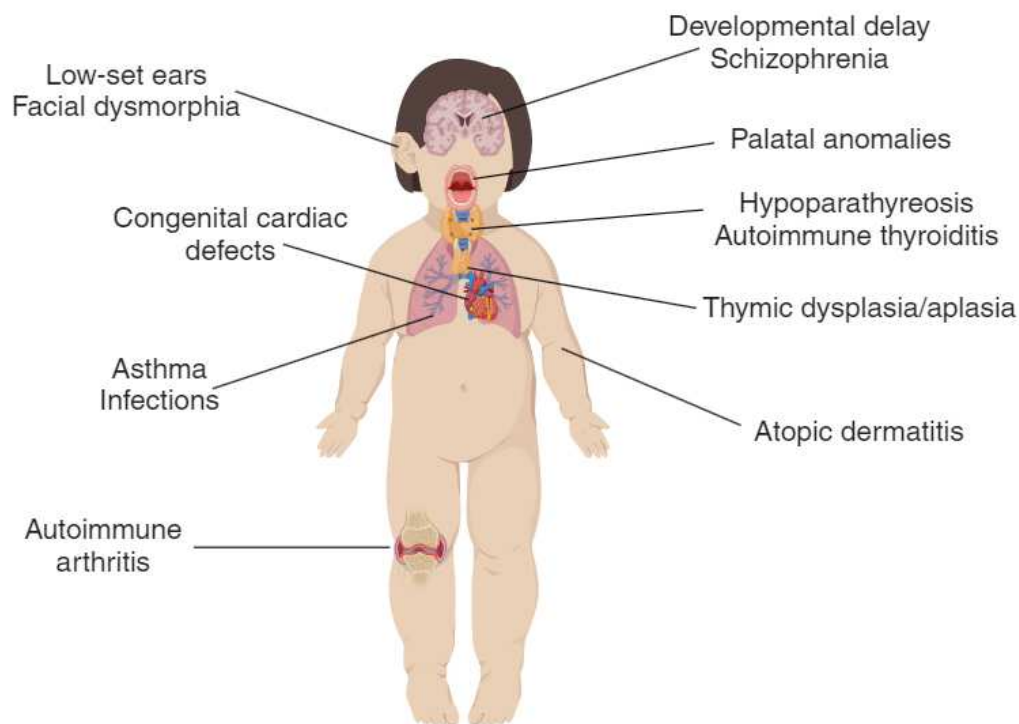


Figure 6 Clinical features of 22q11.2 deletion syndrome. Variable penetrance and phenotypic diversity is characteristic for 22q11.2 deletion syndrome.

In those patients, in whom the focus is primarily on the immune system, “DiGeorge syndrome” is still frequently used. For example, we can find “DiGeorge syndrome” in the European Society for Immunodeficiencies (ESID) diagnostic criteria for inclusion in the international ESID registry of patients with primary immunodeficiencies. Here, such classification requires the patient to have a verified microdeletion of 22q11 or 10p AND signs of immunodeficiency (e.g. recurrent or severe infections) and/or immune dysregulation, although the exact extent to which the immune must be affected is not defined (ESID 2019).

On the other hand, the database of orphan diseases Orphanet only features the nowadays preferred term, 22q11.2 deletion syndrome (22q11.2DS). This carries no negative emotional charge and does not

single out any particular symptom, which means it can be easily applied to all patients bearing the deletion.

Immunologic features of 22q11.2 deletion syndrome – a stroll through the last 20 years of research

As briefly alluded to above and discussed more in depth in my dissertation, the principal cause of immune defects in 22q11.2DS/DiGeorge syndrome is the impaired genesis of the thymus. This organ arises antenatally from the third pharyngeal pouch, the development of which is drastically impaired by the genes missing in these patients. As the parathyroid glands develop from the same primordial structure, they are also affected, leading to the characteristic hypocalcaemia. Further disruption of midline structures through aberrant organ formation and migration results in cardiac defects and velopharyngeal insufficiency.

The original description of DiGeorge syndrome focused on the connection between missing thymus and impaired/missing cellular immunity in patients with severe lymphopenia or even complete absence of lymphocytes. However, we now know that these patients with so called “complete DiGeorge syndrome” represent less than 1% of 22q11.2 deletion bearers (Sullivan 2019), whereas most patients instead only suffer from somewhat decreased T cells – this much more common condition is labelled “partial DiGeorge syndrome”.

Therapy of complete DiGeorge syndrome/22q11.2DS with severe combined immunodeficiency

This distinction dictates the therapeutic approach best tailored for each patient. While patients with partial DiGeorge syndrome may require only transient supportive care with prophylactic antibiotics or other therapies, those with complete DiGeorge syndrome have no T cells and are at high risk of life-threatening infection. Transplantation of hematopoietic stem cells (HSCT), the cornerstone of severe combined immunodeficiency (SCID) treatment, is ineffective in these patients, as their bone marrow and precursor cells function correctly, however, they lack the thymic stroma which to populate and develop in.

In the past, our group has shown that infusion of adult donor lymphocytes is partially helpful in reconstituting cellular immunity in a complete DiGeorge syndrome patient (Janda et al. 2007), leading to some proliferation of donor T cells both *ex vivo* and during an EBV infection. Nevertheless, this procedure also induced strong graft versus host disease and did not repair the original defect, but rather only provided some small buffer amount of T cells to work in stead of the steady stream of physiologically developing lymphocytes.

More recently, we have been able to treat a patient with complete DiGeorge syndrome caused by a 22q11.2 deletion with a thymic transplant, a procedure pioneered already in the 1960s (Cleveland et al. 1968), but refined in the 1990s and 2000s by a team of Luise Markert in Philadelphia and later adopted by a team of Graham Davies in London (M. L. Markert et al. 1999; Davies et al. 2017). This girl was transplanted the thymic stroma (without thymocytes, which were removed during *in vitro* culture) of an immunologically healthy infant undergoing corrective cardiac surgery when she was 3,5 months old (Klocperk and Šedivá 2019) (**Addendum II**). First T cells appeared in her peripheral blood around 5 months (Figure 6) after transplant and now, 6 years later, she is without prophylactic antibiotics, immunoglobulins, with no autoimmunity, fully vaccinated and attending kindergarten.

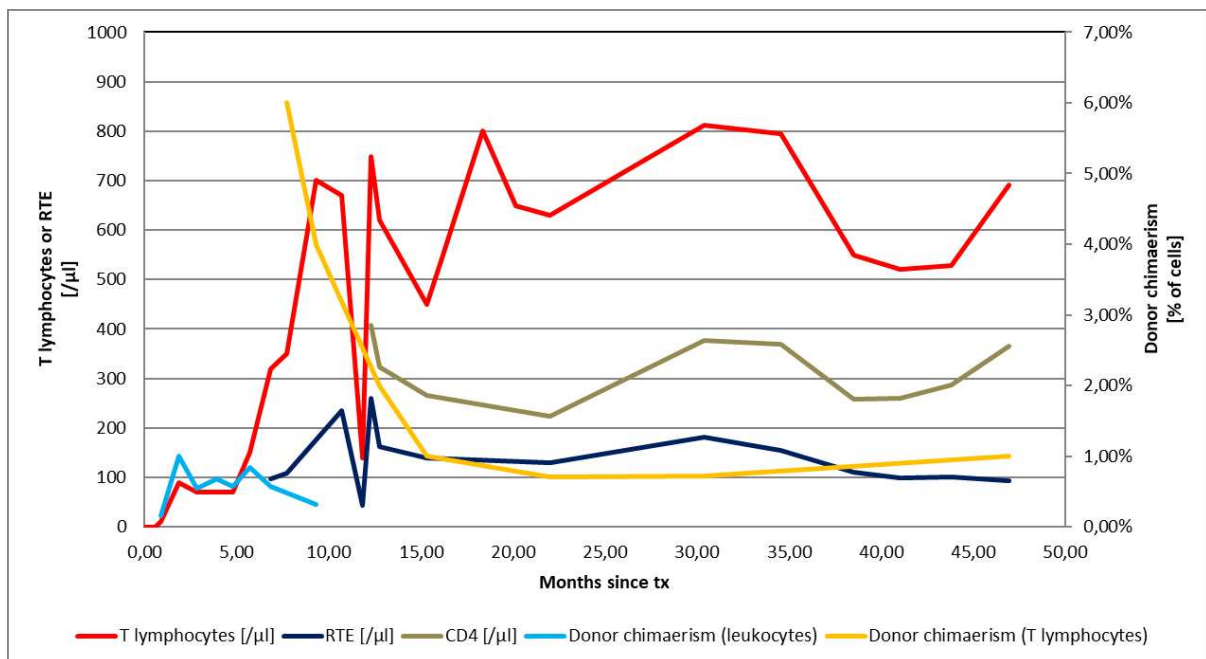


Figure 6 Reconstitution of cellular immunity after thymic transplant. Note the onset of T cell production at 5 months after transplant and a dip at 12 months caused by a viral upper respiratory tract infection. Further note the decrease of donor chimaerism within T cells – this is caused by remaining donor T lymphocytes being “washed out” from the thymic stroma.

We have since then treated another patient with a complete DiGeorge phenotype, however, this patient did not bear the traditional 22q11.2 deletion, but rather a monogenic mutation of TBX1. This gene is known to cause monogenic phenocopy of DiGeorge syndrome and is thus implicated in being one of the critical genes affected by the deletion. This patient was unique by having low-normal T cell counts, however, all of those had memory phenotype, were highly activated and of materno-foetal engraftment origin. They were not efficient in protecting from or clearing infections, but instead caused enteropathy, eosinophilia and skin disease highly reminiscent of the so-called Omenn syndrome, in which a small population of oligoclonal T cells react against the body, rather than

protecting it. Whereas Omenn syndrome is caused by a “leaky” hypomorphic mutation in SCID-related gene like RAG, here, the maternal lymphocytes engrafted trans-placentally and caused a form of graft-versus-host-disease. The patient received anti-thymocyte globulin to eradicate these maternal T cells before transplantation and now, three years after transplantation, is immunologically completely recovered.

Finally, a last patient has been just transplanted in the summer of 2023. This child has been identified as part of the neonatal SCID screening programme, newly implemented in Czechia since January 2022. Interestingly, while he had a characteristic combination of syndromic features – cardiac defect, hypoparathyroidism, no T cells, spinal anomalies, we failed to identify either the characteristic 22q11.2 deletion, or the less typical 10p deletion, or any known monogenic causes of thymic dysplasia (e.g. TBX1 as discussed above, or PAX1, or others). However, his mother is diabetic and during the first trimester of gravidity had elevated glycemia (with glycated haemoglobin A1c between 45-49 mmol/mol). Co-occurrence of maternal diabetes and congenital anomalies, including DiGeorge syndrome, has been described (Novak and Robinson 1994; Digilio et al. 1995), and diabetic embryopathy has been the underlying cause of congenital athymia in upwards of 30% patients who underwent thymic transplant in the Philadelphia centre (M. Louise Markert, Gupton, and McCarthy 2022). Therefore, diabetic embryopathy causing DiGeorge syndrome has now been postulated as a putative diagnosis in this patient. At the same time, one wonders why there are no more athymic patients given the number of (pre)diabetic mothers. The current theory is some level of dose-dependent polygenic susceptibility to hyperglycaemia, in genes such as FGF8 or others (Frank et al. 2002). In this patient, neonatal screening facilitated an extremely early diagnosis before onset of infectious complications. This has been a shared experience across European centres and with the British thymic transplant centre in particular, where neonatal screening lead to earlier referrals of healthier patients and better overall outcome – as we published together recently (Howley et al. 2023).

T cell development in partial DiGeorge/22q11.2 deletion syndrome

In patients with partial DiGeorge syndrome, the immunodeficiency is less pronounced and requires less drastic therapeutic measures, however, it still imprints a particularly interesting phenotype on the developing immune system.

Almost 20 years ago now, our group has published that T cells are particularly low in 22q11.2DS infants under 2 years of age (Šedivá et al. 2005). Nevertheless, these T cells also proliferate rapidly following phytohaemagglutinin stimulation and this leads to gradual relative increase of T cell numbers. This is the so-called process of homeostatic proliferation, which constitutes peripheral proliferation of T cells in an attempt to fill out the pool of total T cells, which are congenitally low due to low thymic output.

IL-7 has been proposed as a cytokine driver of this process in naïve T cells (Boyman et al. 2009) and has been found to be increased in a small pilot study on patients with partial DiGeorge syndrome and T cell lymphopenia (Tantibhaedhyangkul et al. 2009). This leads to skewing of TCR variability and rise of oligoclonality (Pierdominici et al. 2003), but also a skew away from naïve and into memory T cells.

T cell maturation, naïve, memory and TRECs

In our earlier studies we have demonstrated this decrease of naïve T cells through reduced concentration of T cell receptor excision circles (TRECs), circular molecules of DNA excised during VDJ recombination and surrogate markers of naïve T cells (their concentration drops as T cells homeostatically proliferate, as no new TRECs are produced when a single mature T cell divides, as no new VDJ recombination events take place) (Froňková et al. 2014). This test is now the cornerstone of neonatal SCID screening, as patients with no T cells in the blood also have no TRECs in dry blood spots – and it enabled the diagnosis of 6 new patients with 22q11.2DS in the past 18 months of Czech neonatal SCID screening programme, including the third thymic transplant patient discussed above.

Follicular helper T cells

Later, we have shown that this expanded proportion of memory T cells in patients with partial DiGeorge syndrome persists throughout childhood and into adolescence using more specific and sensitive flow cytometric techniques (Klocperk et al. 2018) **(Addendum II)**. More interestingly, however, this exploration of developing T cell phenotype revealed that the population of follicular helper T cells is also significantly increased. Follicular helper T cells are a specific population expressing the chemokine receptor CXCR5 which guides their localization into secondary lymphatic organs along the CXCL13 gradient as produced by follicular dendritic cells (C. S. Ma et al. 2012). These follicular helper T cells are potent producers of IL-21 and crucial in supporting B cell development and class-switch from IgM-producing naïve into IgG/A/D/E-producing switched memory B cells. Their proportion was increased in DiGeorge syndrome patients, regardless of age, although there was a trend towards most substantial increase in the first 10 years of age. The functional impact of this increase is currently unclear, although a subject of further study – one of the currently ongoing projects will assess the function of the 22q11.2DS follicular helper T cells in support of B cells *in vitro*, as methodically demonstrated in one of the project I was able to participate on which tested the function of Tfh in patients with chronic hepatitis (Basho et al. 2021).

B lymphocyte side of 22q11.2DS

Due to the close interconnectedness between T and B cells during adaptive immune response, several groups have previously explored the humoral immunity in patients with 22q11.2DS/DiGeorge syndrome. Most have found no substantial humoral immune deficiency in total immunoglobulin levels,

subclasses or response to vaccination (Junker et al. 1995; Patel et al. 2012; Jawad et al. 2011), although irregularities including high titres were noted by others (Gennery et al. 2002). We have locally shown a very interesting pattern of impaired B cell maturation, with fairly normal B cell subpopulations in patients of 2 years and younger, but an inability to elicit the switch into IgG-producing memory cells which in healthy children dominates the pre-school age (Klocperk et al. 2015) **(Addendum II)**. The persisting abundance of naïve B cells was supported by elevated levels of BAFF (see section above – [Too much of a good thing – MIS-C, Hck, Caspase 8](#)). This seems to contradict the finding of high Tfh cells. However, we speculate that these increased Tfh cells provide insufficient and impaired support for B cells, as reflected in the low proportion of switched memory B cells, but in those B cells they DO support may elicit a dysregulated response, which we regularly note in a subset of patients as a trend towards hypergammaglobulinaemia, as also documented in the 2015 paper. Notably, this trend has been previously shown by others (Gennery et al. 2002) and there is a recognized propensity of 22q11.2DS patients towards the development of immune pathologies mediated by antibodies – allergies and autoimmune disorders (Jawad et al. 2001; Cancrini et al. 2014; Sullivan et al. 1997).

We have also shown a very interesting and as of yet unexplained lack of marginal zone-like (MZ-like) B cells in 22q11.2DS patients (Klocperk et al. 2015) **(Addendum II)**. These are a population of B cells which provide T cell-independent antibody response through generation of “natural antibodies” – they may be activated through ligation of toll-like receptors or B cell receptor cross-linking and produce generally lower-affinity IgM antibodies. This decrease of MZ-like B cells was accompanied by a concomitant decrease of anti- α -galactosyl IgM antibodies, a common autoantibody against food-borne carbohydrate moieties. Where we expected perhaps a compensatory increase in T cell-independent B cell varieties we instead found a surprising lack thereof. Might this be somehow influenced by the follicular helper T cell dysregulation?

Regulatory T cells as (lacking) guards against autoimmunity?

While we have not seen any difference between the proportion or phenotype of Tfh in patients with and without allergy or autoimmunity (Klocperk et al. 2018) **(Addendum II)**, we considered the role of the thymus in generation of regulatory T cells. Regulatory T cells (Tregs) suppress and regulate (hence the name) immune responses, protecting the body against inflammation-induced organ damage and autoimmunity. Tregs may be centrally derived from the thymus, where they arise from T cells recognizing self-antigens with low to medium affinity (highly affinitive T cells are deleted as part of negative selection, those which don't recognize self-antigens become regular effector T cells), or in the periphery, through incompletely understood mechanisms (presumably when a naïve T cell recognizes an antigen presented on an immature antigen presenting cell, without the secondary signal of

costimulatory molecules and tertiary signals of proinflammatory cytokines). Ten years ago, there was some body of evidence supporting the notion that thymus-derived Tregs can be distinguished from peripheral induced Tregs through the expression of the transcription factor Helios (Dhamne et al. 2013; Sugimoto et al. 2006; Thornton et al. 2010). We have thus tested the proportion of Helios⁺ Tregs in patients with 22q11.2DS, expecting them to be low – however, this turned out to not be the case. Indeed, the proportion of total Tregs to T cells was comparable between patients and controls, and the proportion of Helios⁺ Tregs was also similar (Klopper et al. 2014) (**Addendum II**) (Figure 7).

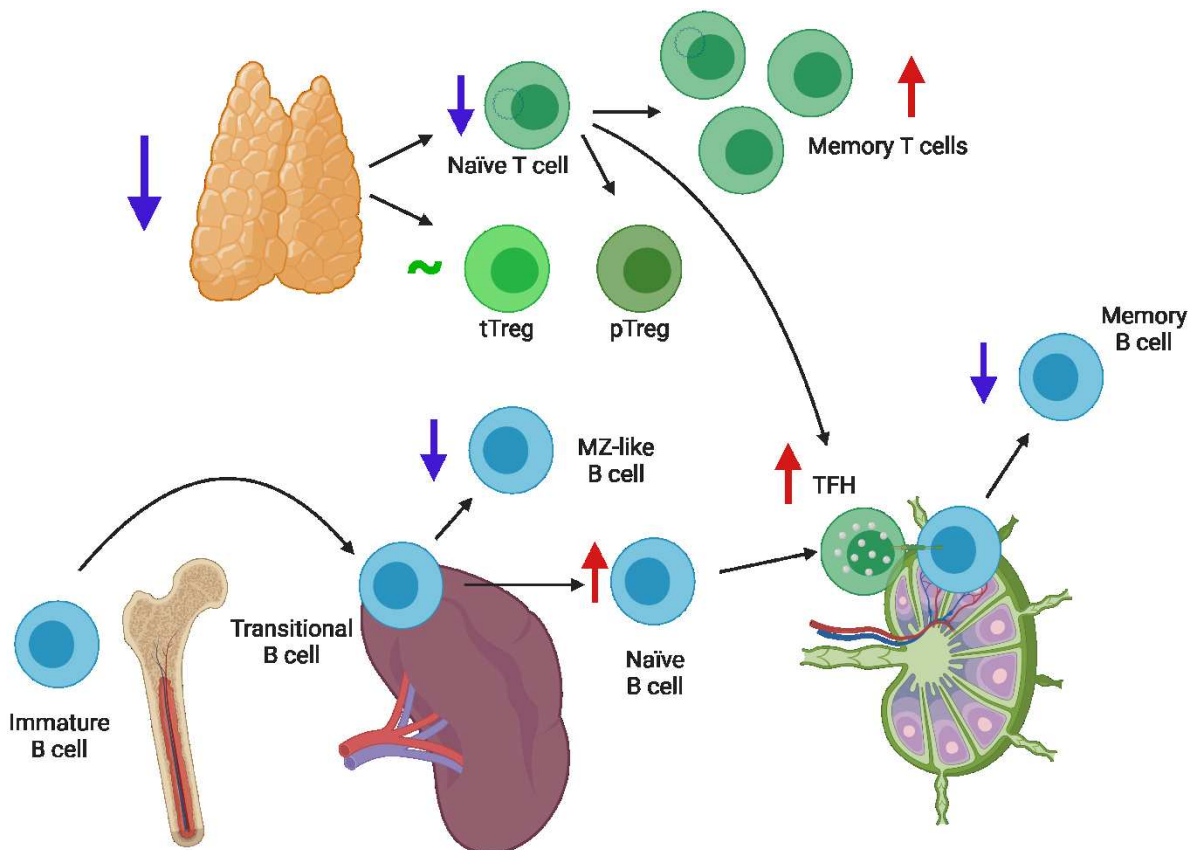


Figure 7 Overview of changes within the adaptive immunity of 22q11.2DS patients. The low thymic output results in low naïve T cells and high memory T cells acquired through homeostatic proliferation. Despite an increase in follicular helper T cells, these fail to provide sufficient help to B cells, resulting in a block in B cell maturation.

Worlds of memory

The persistent proliferation exerts a large pressure on T cells to maintain correct functionality. We have already discussed that as its result, naïve T cells acquire memory phenotype. However, the division into naïve and memory T cells offers little granularity and biological insight.

Indeed, a subset of naïve T cells representative of the most nascent T cells which just recently emigrated from the thymus – recent thymic emigrants (RTEs) can be distinguished through expression

of CD31 and protein kinase 7 (Haines et al. 2009). RTEs proliferate less readily, produce fewer cytokines and – in mice – retain expression of Rag2 (Cunningham, Helm, and Fink 2018). They are also more susceptible to anergy and clonal deletion when recognizing an antigen in a non-inflammatory milieu, thus prolonging/completing negative selection even outside the thymus, expanding the spectrum of tolerated antigens. We and others have shown that RTEs are – as expected – also low in 22q11.2DS patients (Smetanova et al. 2022) **(Addendum II)**.

Further, different subtypes of memory T cells which have undergone contact with their cognate antigen fill distinct niches (Romero et al. 2007) – commonly recognized subsets include central memory (CM) T cells which express the chemokine receptor CCR7 guiding them into the paracortex of secondary lymphoid organs, and effector memory (EM) T cells, which lack it. Consequently, CM T cells are thought to be long-lived wardens of immune memory (Willinger et al. 2005), ready to proliferate during antigen re-exposure, whereas EM T cells express the highest levels of cytotoxic molecules and effector cytokines (thus the name), but are located in the periphery and lack the capacity for long-term self-renewal (Federica Sallusto, Geginat, and Lanzavecchia 2004; F. Sallusto et al. 1999). Finally, some effector memory cells with high concentrations of effector molecules may re-express the CD45RA molecule – TEMRAs. The currently most widely adopted paradigm propose a linear progression model from naïve into CM, EM (with further EM1, 2, 3, 4 subset distinguished by gradual loss of CD27 and CD28 costimulatory molecules) and finally the terminal effector TEMRA subsets (van Aalderen et al. 2017).

We have shown that in paediatric 22q11.2DS patients, all three memory subtypes – CM, EM and TEMRA CD4 as well as EM CD8 T cells – are expanded, with EM and TEMRA being affected slightly more strongly (Smetanova et al. 2022) **(Addendum II)**.

Further distinctions arose especially with the advent of unbiased high-parametric methods which are perfect for discovery of novel subsets and then inferring and subsequently testing their biological properties. Stem cell memory T cells are a sort of in-between population with naïve-like phenotype, ability to repeatedly self-renew and re-populate the entire spectrum of daughter T cell stages, but also the memory-like expression of CD95/Fas and history of antigen exposure, thus sitting in between naïve and central memory cells (Gattinoni et al. 2011, 2017). CD103⁺ resident memory T cells are unique residents of non-lymphoid tissues, where they confer immunologic memory, can initiate production of proinflammatory cytokines (Ariotti et al. 2014) and provide onco-immunosurveillance (Malik et al. 2017), but do not recirculate through peripheral blood (Jameson and Masopust 2018). To my knowledge, these populations have not yet been studied in 22q11.2DS – of particular interest may be

the stem cell memory T cells, given the continuous pressure on T cells in 22q11.2DS to proliferate and self-renew.

T cell proliferation as a driving factor of T cell senescence and exhaustion

As suggested above, continuous proliferation of T cells imposes replication stress on the original naïve T cells – repeated proliferation causes replicative senescence through progressive telomere shortening from naïve into memory and terminal effector cells (Fritsch et al. 2005; Mittelbrunn and Kroemer 2021) – the Hayflick limit. Both naïve and stem cell memory T cells express some levels of telomerase to partially protect themselves from this gradual decline (Ahmed et al. 2016) and a new and exciting study showed that naïve and central memory T cells are in fact able to obtain telomeric DNA via vesicles from antigen presenting cells to achieve the same end (Lanna et al. 2022). Nevertheless, cellular senescence is an ultimately unavoidable hallmark of immune aging (Mittelbrunn and Kroemer 2021). Senescent T cells have reduced proliferation capability and produce inflammatory cytokines – a senescence-associated secretory profile (SASP) (Tchkonia et al. 2013) – this is part of what causes “inflammaging”, the chronic inflammatory milieu which promotes a host of chronic conditions associated with age. We have become interested in the phenomenon of senescence in 22q11.2DS patients and have been the first group to document an increased expression of the best-known senescence-associated molecule CD57 in patient CD4 and CD8 T cells (Smetanova et al. 2022) **(Addendum II)**. While in 22q11.2DS patients this increase is driven by augmented homeostatic proliferation of T cells, others have shown the same result in disorders with intrinsic T cell defect driven by hyperactive phosphoinositide 3-kinase delta (Cura Daball et al. 2018).

While repeated proliferation eventually drives cellular senescence, recurrent antigenic stimulation leads to T cell exhaustion (Wherry and Kurachi 2015). While this concept has been most explored in chronic viral infection (such as CMV, HIV or hepatitis C) in CD8 T cells, however some indications for CD4 T cell exhaustion also exist (McKinney et al. 2015). T cell exhaustion is a state where effector T cells upregulate inhibitory receptors (most famously PD-1, TIGIT, Tim3, LAG3, KLRG1 or others) and slightly downregulate their effector cytokines and molecules. While biologically this process evolved to protect the host against rampant inflammation-induced tissue damage during chronic infections (Pawelec 2019), it's unfortunate side-effect may be the failure to clear the pathogen (Bensch et al. 2010). A balance between “pathogen” clearance and host protection is also of vital importance in defence against tumours, where tumor-infiltrating lymphocytes (TILs) frequently acquire exhausted phenotype with upregulated inhibitory receptors and as such as too suppressed and ineffective at clearing off the cancerous cells. Therapies which aim to overcome this fail-state now exist, chief among which is the checkpoint inhibition therapy targeting the PD-1 and CTLA4 molecules (Topalian et al.

2012), which reinvigorates the exhausted T cells in their fight against the cancer cells and for which Tasuku Honjo and James P. Allison were awarded the 2018 Nobel Prize in Physiology and Medicine. Similar approaches can be taken in chronic viral infections, where checkpoint blockade provides a burst of virus-specific CD8 T cells expressing TCF1 (Utzschneider et al. 2016; Wieland et al. 2017).

Since repeated stimulation with infectious antigens are a hallmark of patients with primary immunodeficiencies and since the repeated stimulation of T cells towards proliferation may confer similar risks for the long-term quality of T cell function as chronic antigenic stimulation, we explored the landscape of T cell exhaustion in patients with 22q11.2DS. Indeed, we found an upregulation of exhaustion-associated markers including PD-1 and KLRG1 especially in patients under 5 years of age (Smetanova et al. 2022) **(Addendum II)**. Patient T cells also expressed a combination of transcription factors Tbet and Eomesodermin that is characteristic for T cell exhaustion, particularly in combination with PD-1 and KLRG1. Interestingly, there was a higher expression of these markers in a subcohort of patients with more severe immunophenotype and lower total and naïve T cell counts – this discovery has been a trend in 22q11.2DS research in recent years, where patients with low CD4 T cell counts (usually <400/ μ l) seem to have more autoimmune complications (Giardino et al. 2019) and generally worse prognosis. This again illustrates the phenotypic breadth of 22q11.2DS and suggests the suitability of patient stratification according to their (immune) phenotype.

When we employed a more granular analysis of phenotypes within the different T cell naïve/memory subsets, the pattern of differences between patients and healthy donors shifted, with PD-1 expression being higher in naïve CD8 T cells of older (5-20 years old) but not young (<5 years) patients, and KLRG1 showing a drastic increase in older patient naïve CD4 T cells, but comparable expression in all subsets of young patient CD4 T cells (Smetanova et al. 2022) **(Addendum II)**. The interpretation of these results was not entirely clear, however, I suspected that a combination of transcriptional, epigenetic and functional single-cell analysis may provide explanations on the developmental fates of T cells in the context of impaired thymic output.

Transcriptional and functional impact of thymic dysplasia and homeostatic proliferation

To this end, in our latest project we focused on adolescent patients with 22q11.2DS and employed modern strategies and approaches to interrogate the function and developmental fate of their T cells (Vladyka et al. 2023) **(Addendum II)**. First, we were able to recapitulate previous results of low RTE, naïve and high CM and EM T cells – strikingly, the difference was greatest in the CM population, which fits the narrative that CM are capable of repeated proliferation and reconstitution of the whole T cell compartment, which is something 22q11.2DS patients require due to lower thymic output.

Next, we found that the proportion of Th1 cells – characterized by expression of CXCR3 but lack of CCR6 – was significantly higher in patients compared to controls, including in the differentiated memory compartment. Exploring the functional impact of this change, we found that isolated patient T cells produced significantly higher levels of the characteristic Th1 cytokine IFN- γ , but also IL-2, IL-10, IL-21 and TNF- α , but not the Th2 cytokine IL-4. The increase in IL-21 is particularly notable, as it may be the result of the elevated proportion of follicular helper T cells which was discussed previously, but which we also noted in this cohort. The Th1 bias was also notable on the transcriptional level, where RNA sequencing performed on isolated bulk T cells revealed IFN- γ mediated signalling to be one of the top dysregulated pathways in patient cells.

We then used spectral cytometry to interrogate the function, surface phenotype and expression of transcription factors in each analysed cell, with the goal of specifying which population of cells is particularly responsible for the Th1 bias, revealing perhaps previously unsuspected culprits and obtaining higher granularity than was offered in previous studies. Successfully, an unsupervised clustering analysis identified 16 distinct clusters of T cells (rather than the 9 populations we evaluated with Boolean gating), several of which were differentially represented in patient and healthy cells – in particular, CD8 cells with senescent and exhausted phenotype (CD8⁺CD62L⁻CD27⁻CD28⁻TCF1⁻TOX⁺CD57⁺) were present in patients but virtually absent in all healthy age-matched controls.

The phenotypic differences between patient and healthy bulk CD4 and CD8 T cells were substantial, with higher expression of inhibitory molecules PD-1, TIGIT, Tim3, 2B4, as well as the cytotoxic effector molecule Granzyme B, proinflammatory TNF- α , IFN- γ , IL-2 and as expected, the marker of cellular proliferation, nuclear protein Ki67. We also documented a conserved upregulation of CD95/Fas in patient naïve T cells, which is supportive for the role of stem cell memory T cells in T cell repopulation (similar to the increase in central memory T cells, as discussed above). Vitally, however, the granular analysis within individual memory stages enabled by the high-parametric single-cell spectral cytometry showed that IFN- γ production is similar between patient and healthy cells of comparable developmental stage and that, most likely, the increase in IFN- γ production and transcriptional signature is driven by the expanded population of highly developed cells, rather than a particularly strong intrinsic bias.

Finally, we tested the effect of the IL-7 on patient and healthy T cells – IL-7 is a potent stimulator of T cell proliferation and has been tested therapeutically in idiopathic T cell lymphopenia (<https://clinicaltrials.gov/ct2/show/NCT00839436>) and sepsis-induced lymphopenia (<https://clinicaltrials.gov/ct2/show/NCT02640807>), so we considered the option of it being useful in replenishing the T cell pool in high-risk DiGeorge syndrome patients with particularly low numbers of

T cells. However, the results of the cell cultures showed that a 72-hour stimulation with IL-7 resulted in the loss of naïve clusters and expansion of terminal effector TEMRA cells, which are the main producers of IFN- γ . Even within memory compartments, IL-7 lead to upregulation of exhaustion-associated transcription factor TOX, the pro-apoptotic CD95/Fas receptor and loss of progenitor transcription factor TCF1. IFN- γ production was further upregulated in both HD and 22q11.2DS memory/effector cells through IL-7. As such, while IL-7 may lead to alleviation of patient lymphopenia through upregulation of Ki67 and promotion of cellular replication, it would also further accentuate the Th1 bias (Figure 8).

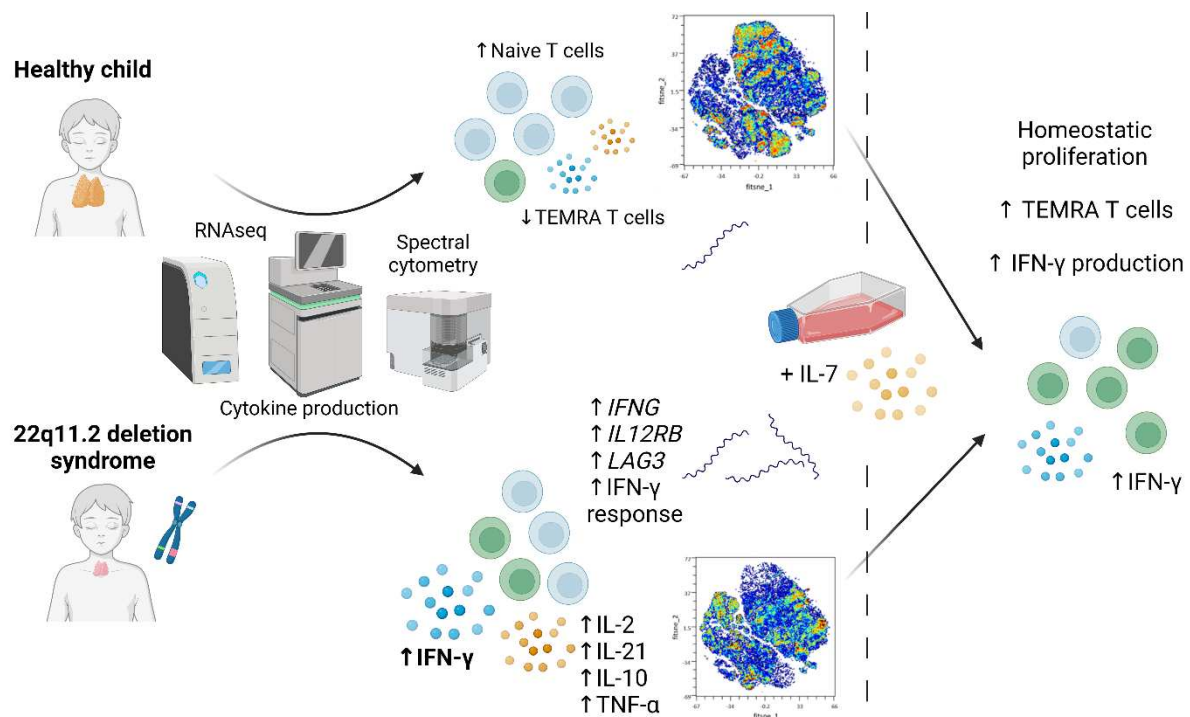


Figure 8 T cell maturation and Th1 bias is shaped by IL-7 driven homeostatic proliferation in 22q11.2DS patients. Note the elevated production of all cytokines by the enlarged population of memory and effector T cells, which is further augmented in both HD and 22q11.2DS patients by culturing the cells with IL-7 which promotes homeostatic proliferation.

This project was the culmination of our long-standing interest in the pathology induced by thymic dysplasia in 22q11.2DS, married with the acquired skillsets of cell isolation, cell cultures, analysing RNA-sequencing data, designing and optimizing high-parametric spectral cytometry data with subsequent non-trivial bioinformatic analysis.

TRANSLATING LESSONS ACROSS THE FIELD OF IMMUNOLOGY

Is CVID common or combined variable immunodeficiency?

Hopefully the above sections have illustrated that immune system needs to work in balance and both too little and too much strength/activity is likely to cause pathology.

Another important lesson which has been highlighted in recent years has been the fact that even in diseases in which the bulk of pathogenicity has previously been ascribed to a single cellular population (e.g. Th17 cells in AD-HIES discussed above, or T cells in thymic dysplasia syndromes like 22q11.2DS), when one looks closely enough, differences may also emerge in other cell lineages (e.g. the affected B cell compartment in 22q11.2DS), all of which may in fact contribute (perhaps to a lesser or as-yet unappreciated extent) to the disease's pathogenesis and clinical features. These discoveries are particularly likely to arise when scientists discuss and share their experiences and findings across specialties or particular fields of interest, fostering collaboration and broadening the horizons of each other.

A perfect example of this are the common variable immunodeficiency (CVID) projects I've worked on with the German group of Prof. Klaus Warnatz. CVID is the most prevalent symptomatic primary immunodeficiency, with an estimated prevalence of 1:25-50,000. It's classified as a predominantly antibody deficiency (Tangye et al. 2022), hallmarked by absence of serum IgG and either IgA or IgM, and lack of response to either polysaccharide or protein vaccination (Bonilla et al. 2016). Caused by a variably profound deficiency of B cells, which can range from their almost complete absence to – most commonly – impaired class-switching and maturation, this antibody deficiency characteristically presents in patients over 4 years of age with recurrent sinopulmonary infections. Patients are usually treated with immunoglobulin replacement therapy, which largely abrogates the disease phenotype. However, in about one third of patients, the disease has a more complex and adverse phenotype, with a host of non-infectious complications, including non-infectious enteropathy, autoimmune cytopenias, interstitial lung disease, granulomatous disease, hepatopathy or more (Resnick and Cunningham-Rundles 2012). Patient with complex CVID (CVIDc) have much worse overall prognosis than those with infection only CVID (CVIDio) (Resnick et al. 2012; Chapel et al. 2008), require more frequent monitoring, screening for organ damage, and usually require some level of immunosuppressive therapy targeted at the dysregulated immune system.

Inspired by the knowledge of T cells from my previous work with 22q11.2DS and following previous works of the same group, which were indicative of a Th1 shift in patients with CVIDc (Unger et al. 2017),

I wondered if the T cell compartment in CVID may contribute to the disease pathology, especially in patients with complex disease – CVIDc, through dysregulated T cell help.

Follicular CD8 T cells regulate and interfere with germinal centre reactions

In our first study, we focused on the biology of the lymph node in CVID patients with generalized lymphadenopathy. Therein we found an aberrant increase in the population of CD8 T cells bearing the chemokine receptor CXCR5, homing them into the lymphatic follicles, as well as another intermediary population of CD8^{low} follicular helper T cells (Klocperk, Unger, et al. 2020) **(Addendum II)**. Follicular CD8 T cells are a relatively recently described T cell subpopulation which has been observed in patients with chronic viral disease such as HIV, in whom they curtail viral replication within the follicles (Leong et al. 2016), and in the setting of autoimmune disease, where they support the autoimmune switching of B cells (Cho et al. 2012; Valentine et al. 2018). As such, it seems that these cells can have both protective and detrimental effects. In patients with CVIDc, we noted that follicular CD8 T cells had surface and transcriptomic phenotype suggestive of advanced differentiation and exhausted status, with the expression of PD-1, TIGIT, CD57, *HAVCR2* (coding Tim3) or *CTLA4* (cursive denotes mRNA gene expression, proteins in regular script). Interestingly, *IL10* was also increased, compared to healthy donor follicular CD8 T cells, both transcriptionally and on cytokine level, suggesting that these cells also have a regulatory function. It has been proposed and we support the notion, that these cells may not be *bona fide* exhausted (Valentine and Hoyer 2019), but instead have a regulatory phenotype with some cytotoxic abilities, regulating – or in the case of CVID, interfering with – the germinal centre T-B interactions. While working with tissue samples can be challenging – not only due to their scarcity, but also due to unique methodologic challenges – it can provide insight simply not apparent in peripheral blood.

Exhausted CD8 T cells characterize complications in CVIDc

Following this description of pathogenic CD8 T cells in a previously dominantly B cell-driven disease, we focused on evaluating whether T cell pathology correlates with other clinical complications and can potentially be used a marker for patient risk-stratification. Building on previous experience of the Warnatz group and their finding of Th1 CD4 T cell bias in patients with CVIDc mentioned above, I wanted to explore the CD8 T cell niche in greater detail. While CVID is a very heterogeneous disease, the Centre for Chronic Immunodeficiency in Freiburg follows one of the most robust cohorts of CVID patients, allowing us to evaluate meaningful numbers of patients with each of the disparate clinical features (such as autoimmune cytopenias, enteropathy, interstitial lung disease, granulomatous disease and others).

Together, we showed that both infection only (CVIDio) and complex (CVIDc) patients have a predominance of CD8 T cells, with a skew away from naïve and into memory stages (Klocperk, Friedmann, et al. 2022) (**Addendum II**). By using 40-parameter mass cytometry we were able to present a temporal-developmental order of both basic memory populations (recapitulating the naïve → central memory → effector memory → TEMRA trajectory). However, the higher resolution and modern bioinformatic approaches allowed us to dissect these larger and more crude populations into 20 unbiased analysis-identified cellular clusters, which map onto the whole developmental trajectory of a CD8 T cell but provide much higher granularity for analysis. We saw a significant reduction of naïve-like clusters, but interestingly, a stark increase in clusters with highly differentiated and exhausted phenotype, which were characteristic for CVIDc.

Since mass cytometry is great for scientific discovery, but its cost, biomaterial requirements and especially analysis difficulty limit its use in routine clinical practice, we then found a simplified surrogate gating strategy which could be used in >10-colour flow cytometry setting and extracted the basic biology identified in the mass cytometry-identified dysregulated clusters. We were then able to show that “Cluster 8/12-like” CD8 T cells with exhausted phenotype (CD127⁺PD-1⁺TIGIT⁺CD28⁻) were able to sensitively and specifically separate CVID patients with interstitial lung disease from those with other or no non-infectious complications, whereas a ratio of TIGIT⁺/CD127⁻ cells was highly predictive for autoimmune cytopenias and CD38⁺ activated CD8 T cells were associated with non-infectious diarrhoea but not with other complications.

While prospective longitudinal study on a separate cohort would be highly valuable to verify the universality and transferability of these results, I believe they already demonstrate how modern scientific approaches can extract clinically valuable information and help translate it into better diagnostic or therapeutic care for patients.

T LYMPHOCYTES AS KEY PLAYERS IN INBORN ERRORS OF THE IMMUNITY

Despite – and partly because of – the advances in our understanding of immunology which highlight the previously underappreciated roles of various cell types in immune disorders, T cells remain both the central coordinators of most and end effectors of many immune reactions.

Focusing on primary immunodeficiencies, we have shown that T cell dysfunction (both hyper and hypoactive) results in disease (e.g. STAT signalling disorders, hyper-IgE syndromes).

Further, we have repeatedly and in incremental detail shown that in 22q11.2 deletion syndrome, the dysplasia of the central organ of the T cell, the thymus, results in an aberrant pattern of T cell maturation, which through homeostatic proliferation skews them heavily in favour of memory stages, towards upregulation of exhaustion-associated inhibitory receptors and increased production of proinflammatory cytokines.

We have shown that modern methods enable high-resolution analysis which provides insight into T cell biology, such as smaller populations of highly differentiated cells which are characteristic for the disease.

We have also demonstrated that aberrant T cell maturation cascades into secondary changes within the humoral immune compartment.

We have been able to show that human thymus transplantation successfully reconstitutes the immune system in a patient with congenital thymus aplasia and that neonatal screening is an extremely helpful tool in identifying patients with inborn disorders of the T cells.

Even in diseases not primarily pathophysiologically connected with dysfunctional T cells, such as common variable immunodeficiency, we have shown that T cells are substantially dysregulated and that their phenotype correlates strongly with disease phenotype and may be used in patient stratification and disease prognosis.

With the advent of novel methods and understandings of the interplay between the components of the immune system, there is more work to be done on the role of the thymus and the role of T cells in immune disorders than perhaps ever before.

Prague, 10.10.2023

MUDr. Adam Klocperk, Ph.D.



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ADDENDUM I: LIST OF PUBLICATIONS

Publications in which the applicant is listed as first, last and corresponding author are underlined.

22q11.2DS

1. Klocperk A, Grecová J, Sišmová K, Kayserová J, Froňková E, Sedivá A, et al. Helios Expression in T-regulatory Cells in Patients with di George Syndrome. J Clin Immunol 2014;34:864–70.
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CVID

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TLR8/7

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HCK

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Other

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ADDENDUM II: SELECTED PUBLICATIONS BY THE AUTHOR