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Review

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Review

The ups and downs of STAT3 function: too much, too little and human immune dysregulation

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Summary

The *STAT3* story has almost 30 years of evolving history. First identified in 1994 as a pro-inflammatory transcription factor, *Signal Transducer and Activator of Transcription 3* (STAT3) has continued to be revealed as a quintessential pleiotropic signalling module spanning fields including infectious diseases, autoimmunity, vaccine responses, metabolism, and malignancy. In 2007, germline heterozygous dominant-negative loss-of-function variants in *STAT3* were discovered as the most common cause for a triad of eczematoid dermatitis with recurrent skin and pulmonary infections, first described in 1966. This finding established that STAT3 plays a critical non-redundant role in immunity against some pathogens, as well as in the connective tissue, dental and musculoskeletal systems. Several years later, in 2014, heterozygous activating gain of function germline *STAT3* variants were found to be causal for cases of early-onset multiorgan autoimmunity, thereby underpinning the notion that STAT3 function needed to be regulated to maintain immune homeostasis. As we and others continue to interrogate biochemical and cellular perturbations due to inborn errors in *STAT3*, we will review our current understanding of STAT3 function, mechanisms of disease pathogenesis, and future directions in this dynamic field.

Keywords: inborn errors of immunity; STAT3; human immunity; immunodeficiency; immune dysregulation

Abbreviations: AD-HIES: autosomal-dominant hyper-IgE syndrome; DNAB: DNA binding domain; GAS: gamma interferon-activated sequence; GOF: gain of function; IEI: inborn errors of immunity; IFN: interferon; IHCA: inflammatory hepatocellular adenoma; iNKT: invariant natural killer T; JAK: Janus Kinase; LGL: large granular lymphocytic leukemia; LOF: loss of function; MAIT: mucosal-associated invariant T; pSTAT3: phosphor-STAT3; Treg: T regulatory cells; T_H: T helper; T1DM: type 1 diabetes mellitus.

STAT3 in the JAK/STAT pathway

Human immune responses require a complex, coordinated amplification of molecular processes to recognize and destroy harmful pathogens. The Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway has long been understood as a key paradigm in responding to, maintaining and amplifying cytokine signals in various immune contexts to provide a robust and balanced immune response. STAT3 is a key transcription factor in the JAK/STAT signalling pathway, contributing to both innate and adaptive immune responses. The *STAT3* locus encodes a 24-exon gene that is broadly conserved through evolution since the teleostean tetrapod branched off 450 million years ago, suggesting its importance in non-redundant biological processes (Figure 1, [1]). Germline deletion of *Stat3* is embryonically lethal in mice [2]. Similarly, targeting the *Stat3α* isoform (which accounts for 95% of STAT3 expression) is perinatally lethal in mice [2]. Extensive studies in mice and humans have established that STAT3 is activated by cytokines belonging to the common gamma chain (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21), the gp130 (IL-6, IL-11, IL-27, IL-35, IL-39, cardiotrophin-like cytokine factor 1, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor), and IL-10 (IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29) receptor families as well as type I and II interferons (IFNα, IFNβ, and IFNγ), IL-12 and IL-23, macrophage and granulocyte colony-stimulating factors, Fms-like tyrosine kinase 3 ligand and hormones such as epidermal growth factor, fibroblast growth factor, growth hormone, insulin-like growth factor (Figure 2, [3–5]). However, a degree of redundancy in STAT3 response to these cytokines exists due to the presence of six other STAT family members (STAT1, STAT2, STAT4, STAT5A, STAT5B, and STAT6), each with variable function in specific cytokine-mediated activation of immune and non-immune cells [4, 6].

Following the binding of cytokine ligands to cognate receptors, cytosolic STAT3 is recruited to the trans-phosphorylated receptor and undergoes JAK-mediated phosphorylation to form phospho (p)-STAT3 homodimers, or heterodimers with pSTAT1, pSTAT4, pSTAT5a, or pSTAT5b (Figure 2, [7–9]). pSTAT3 then translocates to the nucleus where it binds an extensive suite of cis-regulatory modules ranging from composite promoter, enhancer and AT-rich chromatin stability sequences (Figure 2, [10, 11]). Like other members of the STAT family, STAT3 homodimers bind to the canonical GAS-like

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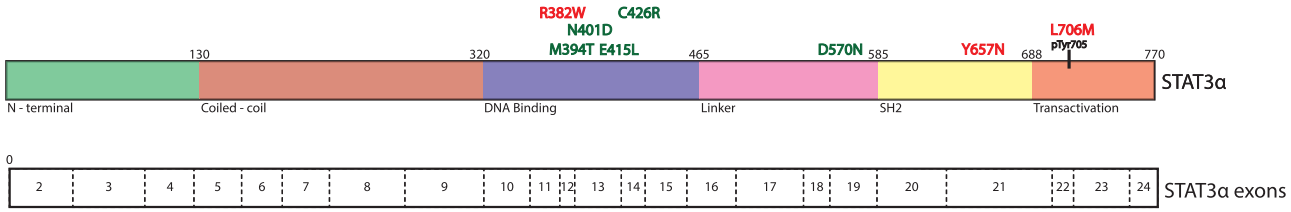

Figure 1. Schematic of functional domain structure (top) and exon map (bottom) of STAT3α isoform. Pathogenic mutations in the DNA Binding, Linker, Src-homology 2, and Transactivation domains have revealed insights into disease pathogenesis in STAT3 LOF and GOF. Red text indicates LOF variants and the green text indicates GOF variants. The canonical cytokine-mediated tyrosine activation site at amino acid residue 705 is denoted by pTyr705

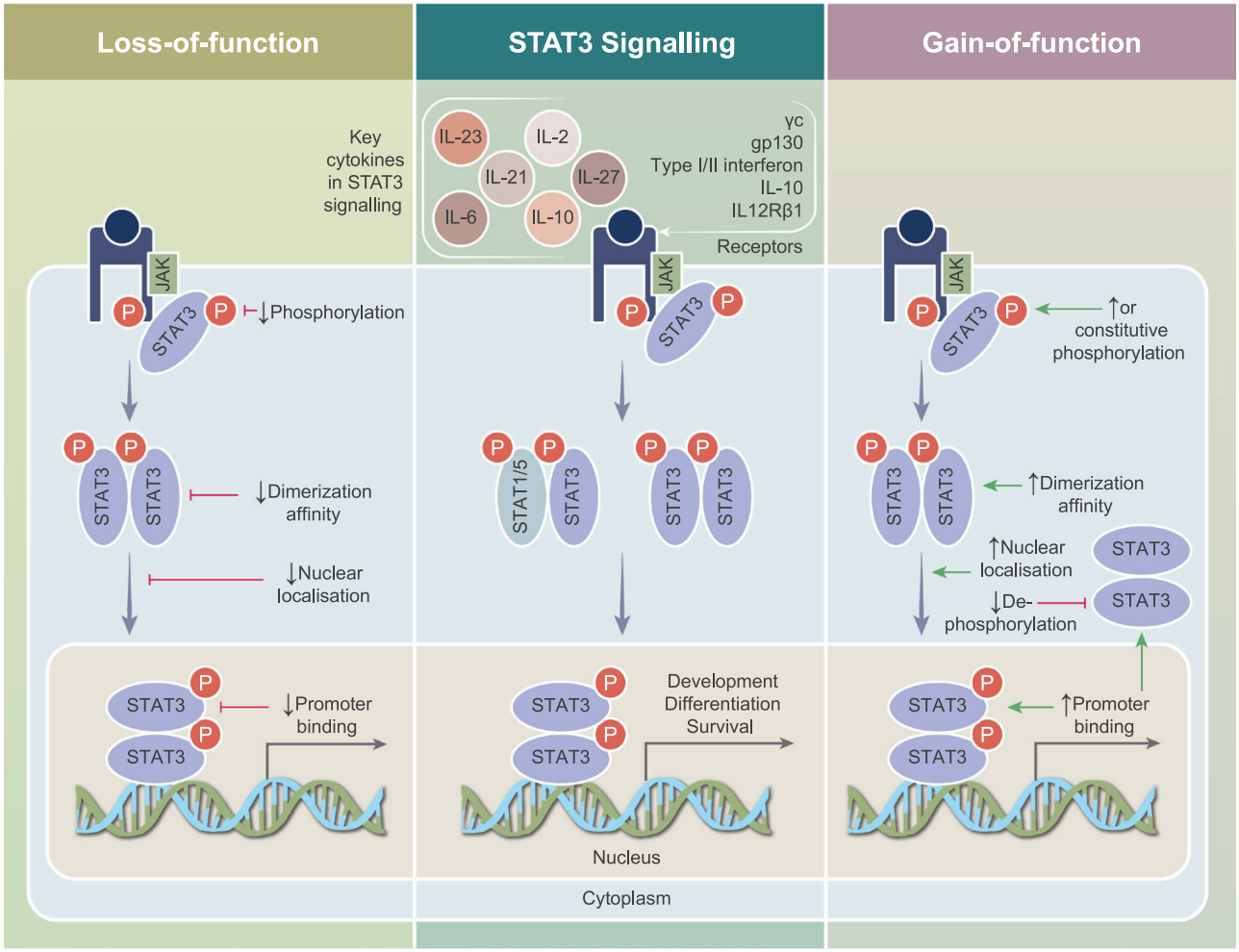

Figure 2. Canonical STAT3 signalling in health and disease. *Middle panel (white):* Ligand binding to its cognate receptor induces trans-phosphorylation of the intracellular domain of the receptor. Latent cytosolic STAT3 is recruited to this activated receptor via SH2-mediated affinity to sites of phosphorylation. Once bound, JAKs phosphorylate the tyrosine reside at amino acid 705 of STAT3 allowing formation of STAT3 homo- and STAT3:STAT1/4/5A/5B heterodimers. Dimers localise into the nucleus where they bind cis-regulatory modules inducing transcription of hundreds of target genes. *Left panel:* LOF variants in STAT3 cause disease via reducing activation, dimerization and promoter binding resulting in a reduction in transcription of target genes. *Right panel:* GOF variants in STAT3 drive disease via increased or constitutive activation, increased dimer affinity, increased nuclear localisation, increased affinity to promoter modules and deceased de-phosphorylation (increased nuclear retention) resulting in an increase in transcription of target genes

motif (gamma interferon-activated sequence, TTCnnnGAA), a variable cis-regulatory module present in hundreds of target genes [11]. Moreover, STAT3 has been described to bind with high affinity to the c-fos promoter (M67 cis-inducible element, TTCCCGGAA) in homodimeric conformation, but also postulated to have modified binding affinity in heterodimeric conformation, the sequences and biological impact of which is poorly understood [12, 13]. In general, STAT3 regulates the transcription of a myriad of genes related to the development, differentiation and survival of haematopoietic and non-haematopoietic cells [14]. As such, tissue and cell-specific cytokine receptor and JAK/STAT pathway regulation as well

as chromatin accessibility and STAT3 dimerization heterogeneity are postulated as mechanisms to explain the pleiotropic ability of STAT3 to underpin gene regulatory networks.

Inborn errors of STAT3 signalling causing human disease

The critical role of STAT3 in healthy and pathogenic conditions has been delineated through the characterization of monogenic germline variants identified in patients with inborn errors of immunity (IEI) [15, 16]. Identification of germline pathological variants in other *STAT* genes (*STAT1, STAT2, STAT5B, STAT6* [17–27]), STAT3-activating cytokines (*IL10, IL17F, IL21* [28–30]), and related receptors (*IL2RA, IL2RG, IL6ST, IL7RA, IL10RA, IL10RB, IL12B, IL12RB1, IL23R, IL17RA, IL17RC, IL21R, IFNGR1, IFNGR2* [31–43]), Janus kinases (*JAK1, JAK3, TYK2* [44–48]), and genes encoding STAT3-associated or -induced proteins (*ZNF341, ERBIN, RORC, FOXP3* [49–53]) have defined the non-redundant functions of STAT3. Strikingly, *STAT3* loss-of-function/dominant negative (LOF/DN) and gain-of-function (GOF) variants have been discovered and cause two distinct clinical syndromes [15, 16, 54]. Paradoxically, *STAT3* LOF/DN and GOF variants do not manifest in antagonistic biochemical, cellular, and clinical consequences [55]. In this unique context, dissecting the pathophysiological impact of each disease-causing mutation is essential for understanding such intricate signalling pathways and their roles in host-defence to infection, autoimmunity and immune regulation, vaccine design and malignancy.

Autosomal Dominant hyper-IgE syndrome

Heterozygous LOF/DN *STAT3* variants cause Autosomal-Dominant Hyper-IgE Syndrome (AD-HIES), a rare, multisystemic condition affecting ~1 in 100,000–1,000,000 live births [5, 15, 16]. First described as Job's syndrome in 1966 by Davis and colleagues, and then termed "extreme hyper IgE" in 1972 by Buckley et al., this condition commonly presents in infancy with non-inflammatory or "cold" atopic dermatitis, reminiscent of the boils the biblical character Job was afflicted with [56–58]. As identified from the largest cohort study of 60 patients from a French national survey, STAT3 AD-HIES is defined by recurrent skin disease (93%), impaired inflammatory responses (73%), life-threatening bacterial (100%), and fungal (85%) infections predominantly caused by *Staphylococcus aureus* (94%) and *Candida albicans* (88%) respectively, and incredibly high levels of serum IgE (96%, Figure 3, [59]). A host of non-immune features affecting the musculoskeletal and dental systems, including osteopenia, scoliosis, retention of primary teeth, and abnormalities in connective tissue, are also commonly observed (Figure 3 [5]). These clinical observations confirm the pleiotropic function of STAT3 in human biology. Interestingly, HIES is also a feature of patients harbouring pathogenic biallelic variants in *DOCK8*, *PGM3*, *ZNF341, IL6ST*, *IL6R, TYK2*, or monoallelic variants in *IL6ST, CARD11,* or *CARD14;* however, *STAT3* LOF/DN variants remain the most common genetic aetiology of aberrant IgE levels and atopy [60].

Mechanistically, reduced STAT3 activity due to pathogenic variants in *TYK2*, *IL6ST*, *IL6R*, or *ZNF341*, which encodes a regulator of STAT3 transcriptional activity, provides a clear link to the role that impaired IL-6/STAT3 signalling plays in driving the dysregulated hyper-IgE phenotype. Similarly, some reports have linked DOCK8 function with STAT3 activation, and PGM3 deficiency has been found to impede IL-6-mediated STAT3 signalling, further linking dysregulated IgE production with impaired IL-6/STAT3 function [61, 62]. Thus far, the cause of hyper-IgE due to pathogenic variants in *CARD11* or *CARD14* remains to be determined.

Mechanisms of disease pathogenesis due to *STAT3* LOF/DN variants

Investigating the consequences of LOF/DN variants on STAT3 signalling at the biochemical level enables not only the discovery of key functional and regulatory features but also better comprehension of STAT3-dependent signalling in different cell types and cytokine environments. Variants in the different functional domains of STAT3 have been shown to have different impacts at the molecular level (Figure 1, [63]). For example, missense variants in the transactivation domain (e.g. p.L706M) near the canonical phospho-tyrosine residue (Tyr705, Figure 1) abolished JAK-mediated STAT3 phosphorylation, leading to a reduction in dimerization, nuclear translocation and binding to target DNA sequences (Figure 2). On the other hand, variants in the Src-homology 2 domain (SH2, e.g. p.Y657N) had no effect on phosphorylation, but did reduce the formation of pSTAT3 dimers, thereby decreasing nuclear translocation and minimizing its functional capacity as a transcription factor (Figure 2). Variants located in the DNA binding domain (DNAB, e.g. R382W) do not affect STAT3 activation, dimerization, or nuclear retention, but result in reduced affinity of mutant STAT3 for target gene promoter sequences (Figure 2). *STAT3* variants have also been identified, and validated to be pathogenic, in the linker domain of STAT3; however, the mechanism by which these variants cause disease remains to be determined [64, 65]. Structural protein changes due to genetic perturbation in this domain that causes misfolding could underlie deleterious function in these cases. Two N-terminal LOF variants have been proposed however these need to be tested further to pinpoint these variants as causal of disease [59, 65]. Scarcity of validated pathogenic variants in functional domains other than the DNAB, SH2, or TA domains could be due to historic biases in sequencing panels that miss potentially pathogenic genetic alterations.

Importantly, reduced protein expression has not been observed in driving STAT3 HIES biochemically. Overall, all pathogenic LOF/DN variants identified have been described to reduce cytokine-mediated induction of STAT3 target genes. Indeed, Asano *et al.* studied all known pathogenic *STAT3* LOF variants (143 in total) and found that all mutant alleles follow a mechanism of negative dominance on the wild-type allele, thereby reducing the total pool of functional STAT3 dimers [65]. These studies showed the necessity of carefully assessing the consequences of novel *STAT3* LOF/DN candidate variants on protein function (i.e. phosphorylation, dimerization, nuclear translocation, DNA binding, transcriptional activation). In general, Asano *et al.* used the canonical c-fos promoter (M67 cis-inducible element, TTCCCGGAA) to study DNA-binding and transcriptomic activation in ectopic over-expression system [65]. However, chromatin immunoprecipitation established that pathogenic variants can alter the STAT3 DNA binding site, demonstrating a potential

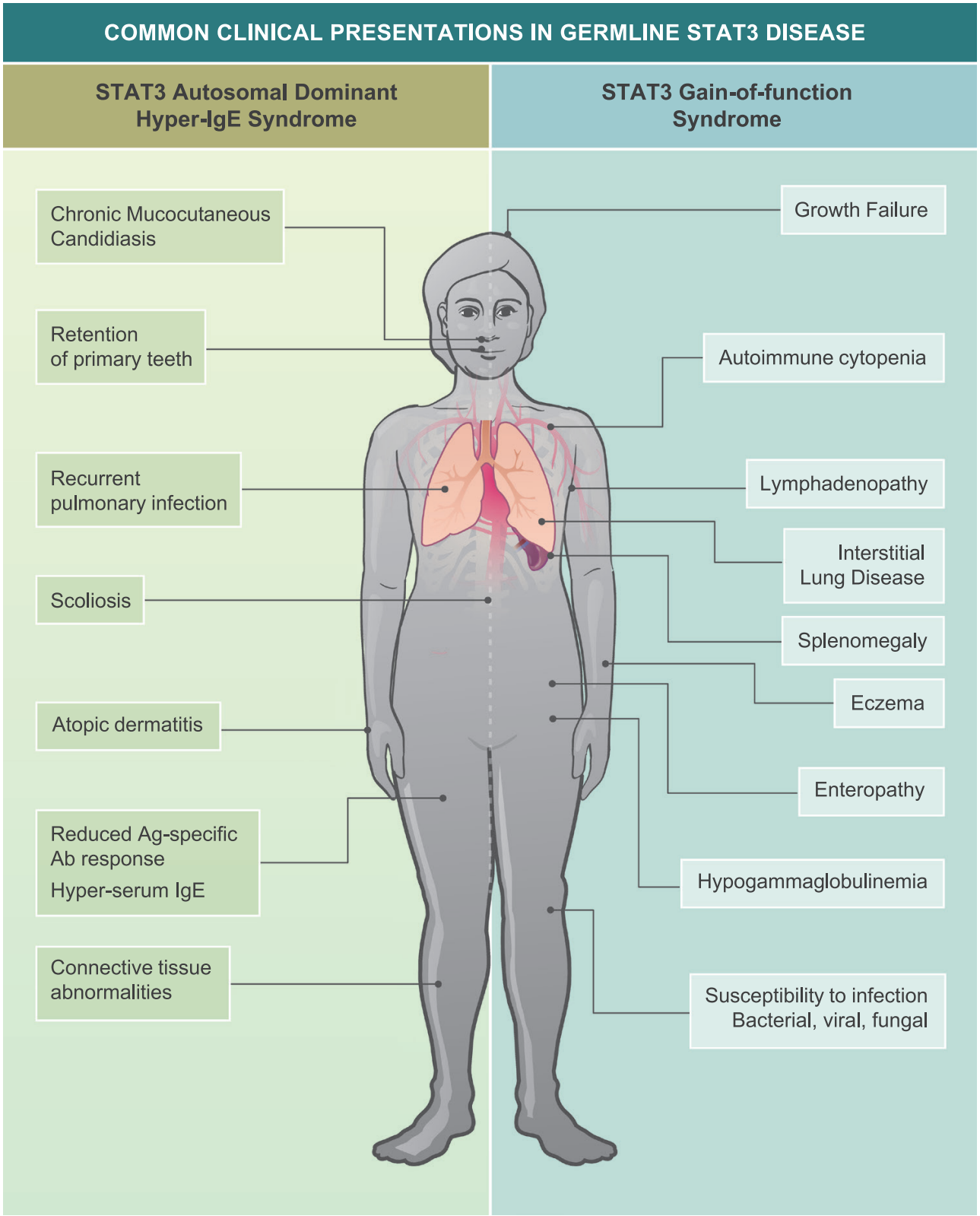

Figure 3. Common clinical manifestations in Autosomal-Dominant Hyper-IgE Syndrome vs STAT3 GOF Syndrome. Despite having the same genetic aetiology, STAT3 AD-HIES and STAT3 GOF Syndrome define two distinct clinical phenotypes

challenge in validating novel variant candidates. Careful consideration should be demonstrated in selecting a binding motif for transcriptional activity assays as well as concluding the pathogenicity of a novel variant [66].

Complexity in elucidating the biochemical mechanism(s) of disease pathogenesis within the STAT3 HIES cohort posit whether a genotype-phenotype relationship exists. Despite displaying an autosomal-dominant (AD) inheritance with

high penetrance, clinical presentation is variable. Heimall *et al*. addressed the question of genotype/phenotype correlation by segregating a STAT3 HIES cohort according to DNAB and SH2 domain variants [67]. Trends were observed for increased mortality and re-activation of varicella-zoster virus infection in the DNAB cohort whereas musculoskeletal defects, sinusitis, and otitis were more common in the SH2 domain cohort. However, as these differences did not reach statistical significance, it would be important to perform similar studies using a larger cohort of patients with a broader range of pathogenic variants. Currently, it remains to be determined whether an individual mutation predisposes to a specific clinical phenotype or presentation.

Impact of *STAT3* LOF/DN variants on immune cell function

Linking functional biochemical defects in *STAT3* LOF/DN variants with their cellular immunological consequences has delineated mechanisms of disease pathogenesis in patients with AD-HIES. These cellular defects predominantly impact $CD4^+$ and $CD8^+$ T cell and humoral B cell adaptive immune responses, which we will focus on here. Crucially, disseminated infection is a primary cause of fatality in this disease emphasising the importance of understanding molecular and cellular perturbations leading to these manifestations [59].

$CD4^+$ T cells

STAT3 regulates the development of the T helper 17 (T_H17) cell population of $CD4^+$ T cells. This is achieved via the induction of the *RORC* gene in response to STAT3-activating cytokines IL-6, IL-21, and IL-23. Human T_H17 cells produce a suite of cytokines including IL-17A, IL-17F, IL-22, and IL-26, which have previously been found to be generated in response to fungal infections and play a key role in antifungal immunity [68]. Defects in T_H17 cells, in terms of both abundance and production of IL17A and IL17F, provide a convincing explanation for the observation of a high proportion (~85%) of STAT3 AD-HIES patients experiencing chronic mucocutaneous candidiasis [38, 68–70]. Impaired T_H17-directed immune responses specifically compromise host defence at mucosal surfaces thereby predisposing these patients to fungal infections at mucosal membranes and barriers, rather than systemic infections.

Interestingly, despite broad cellular immune deficiencies due to *STAT3* LOF/DN, HIES patients tend to have robust viral immunity. The proposed mechanism of this includes both increased production of T helper 2 (T_H2) cytokines as well as a switch to IFN-γ-mediated cell signalling as STAT1 is more readily activated by IFNs in the presence of reduced STAT3 function [68, 71]. Enhanced production of IL-4, IL-5, and IL-13 from T_H2 cells not only supports viral immunity but potentially contributes to the hyper-IgE phenotype by directly promoting B cell IgE class switching, and restraining the function of T_{FH}/T_H17 signals [55].

B cells

Pathogenic *STAT3* LOF/DN variants impair cytokine-induced differentiation of naïve B cells, compromising humoral immune responses in STAT3 AD-HIES [55]. Insensitivity to IL-21, as well as IL-10, via STAT3 LOF/DN results in a reduction in the long-lived memory B cell and antibody-secreting plasmablast cell pool [72, 73]. IL-21 is a potent B-cell growth and differentiation factor and mediates the generation of plasmablasts from naïve B-cell precursors by inducing expression of the requisite plasma cell transcriptional regulators *PRDM1* and *XBP1* [74, 75]. B cell defects in STAT3 AD-HIES are exacerbated by a paucity in T_{FH} cells, which non-redundantly require STAT3 signalling to upregulate *BCL6* and *IL-21* to mediate T_{FH} cell fate commitment and function respectively [76]. Marked reductions in this key population of B-cell helper $CD4^+$ T cells likely contribute to perturbations in humoral immunity, resulting in hypogammaglobulinemia, the resultant susceptibility to *S. aureus* infection, and poor immune responses to vaccines and other infections [76, 77].

Remarkably, the namesake clinical presentation, incredibly high serum IgE levels, remains poorly understood. It appears the exacerbated T_H2 cytokine profile in these patients is alone insufficient to cause hyper-IgE. This is because other monogenic disorders causing enhanced T_H2 cytokine production (variants in *IL12RB1, IFNGR1, RORC, IL21, IL21R, IL10RA*) do not universally present with levels of serum IgE in the same range as STAT3 AD-HIES [55]. Mouse models suggest a B-cell intrinsic mechanism contributes to this phenotype, indicating that STAT3 signalling in B cells negatively regulates Ig isotype class switching to IgE [78]. However, the exact mechanism remains to be elucidated.

$CD8^+$ T cells

In addition to $CD4^+$ T cell defects, a paucity of memory $CD8^+$ T cell populations have been observed in patients with AD-HIES. STAT3 LOF/DN $CD8^+$ T cells express significantly lower amounts of granzyme B in response to stimulation with IL-21, thus potentially dampening their cytolytic potential. However, this defect can be rescued by T cell receptor co-stimulation or IL-2/IL-15. On one hand, these compensatory pathways may explain why susceptibility to viral infection is not a prominent clinical feature of STAT3 AD-HIES. On the other hand, impaired generation of memory $CD8^+$ T cells may underpin reduced $CD8^+$ T cell immune surveillance, resulting in increased episodes of re-activation of herpes virus infections and B-cell malignancy in STAT3 AD-HIES [79, 80].

Unconventional T cells

Of emerging interest in STAT3 AD-HIES is the reduction in unconventional T cell populations, namely mucosal-associated invariant T (MAIT) and invariant natural killer T (iNKT) cells [81]. These subtypes, which comprise up to 15% of peripheral blood lymphocytes, have limited TCR diversity and a high propensity to rapidly respond to stimulation with specific non-polymorphic microbial antigens [82]. In addition to reduced abundance, STAT3 LOF/DN cripples secretion of IL17A and IL17F by these cells suggesting that they could play a role in impaired immunity and immune dysregulation in patients with AD-HIES.

STAT3 GOF syndrome

By 2014, the central role STAT3 played in orchestrating cytokine-mediated immune responses and host defence was clear [6]. Therefore, the discovery that germline *STAT3* GOF variants caused a primary immune regulatory disorder broadly defined by lymphoproliferation, autoimmunity, immunodeficiency, and growth delay was not entirely unexpected. Since then, ~200 patients with *STAT3* GOF variants

have been identified. Recently, Leiding *et al.* have provided an exhaustive natural history of STAT3 GOF syndrome, detailing a global cohort of 191 patients with 72 distinct and validated pathogenic *STAT3* GOF variants [83]. From a clinical perspective, patients most commonly present with lymphadenopathy (75%), splenomegaly (72%), autoimmune cytopenia (67%, mostly due to autoantibodies specific for erythrocytes, platelets, or granulocytes), interstitial lung disease (43%), growth failure (57%), enteropathy (53%, commonly coeliac disease), skin lesions (48%, commonly eczema), and susceptibility to bacterial (59%), viral (44%) and, to a lesser extent, fungal infections (18%, Figure 3, [83]).

STAT3 GOF syndrome has a heterogenous, multisystemic clinical presentation. About 85% of confirmed STAT3 GOF syndrome patients will have at least three separate manifestations of disease, requiring a combination of therapies ultimately reducing the quality of life [83]. There is incomplete disease penetrance across kindreds and variants, suggesting gene modifiers or environmental factors contribute to disease pathogenesis and severity. Incidence is rare, however, a growing number of cohort studies have facilitated the exploration of the biochemical and cellular implications due to these pathogenic activating variants.

Mechanisms of disease pathogenesis in STAT3 GOF

To date, no *STAT3* GOF mutation has been reported to result in protein over-expression, despite STAT3 being known to positively regulate its own transcription. This observation contrasts that observed for some *STAT1* GOF variants which can be pathogenic simply by increasing expression of mutant STAT1 protein [84]. STAT3 GOF variants have been reported to confer hyper-activation through different biochemical mechanisms including enhanced basal STAT3 activity, enhanced cytokine-dependent STAT3 activation, impaired/delayed STAT3 de-phosphorylation, enhanced stability of activated STAT3 homodimers, and increased affinity of GOF STAT3 to DNA binding targets. Despite the increasing number of reports, no association can be drawn between the domain location of the mutation and the biochemical consequence driving increased expression of target genes [85, 86]. In fact, mutational hotspots have been demonstrated to illicit diverse patterns of target gene hyper-activation. For instance, a DNAB hotspot from amino acid position 394-415 contains validated pathogenic variants conferring overactivation via constitutive activation (p.M394T), impaired de-phosphorylation (p.N401D) or enhanced DNA-binding affinity (p.E415L, Figure 1, [85, 86]). Moreover, pathogenic variants have been identified that augment affinity to distinct promoter regions, potentially resulting in the upregulation of some, but not all, target genes. For example, p.C426R and p.D570N have enhanced affinity for the promoter sequence of *SOCS3* but not the *c-fos* promoter sequence that is routinely used in functional assays to validate novel candidate *STAT3* GOF variants (Figure 1, [86]). This presents the possibility that neo-morphic STAT3 functionality is possible in some cases and could contribute to the diverse clinical phenotype observe across the cohort. Therefore, it is critical to rigorously assess and orthogonally confirm the pathophysiological consequences of any *STAT3* GOF candidate variant.

Impact of STAT3 GOF variants on immune cell function

Variants imparting STAT3 GOF signalling will clearly have broad cellular effects due to the ubiquitous expression and pleiotropy of this pathway. The biochemical complexities assumedly have varying impacts according to cell-type and epigenetic state, thereby further complicating our understanding of the mechanisms underlying disease. Here, we describe examples of cellular alterations observed in patients carrying *STAT3* GOF variants, representing important areas of investigation in delineating mechanisms of disease pathogenesis.

One of the most common cellular phenotypes described is lymphoproliferative manifestations [83]. In the T cell compartment, the vast majority of patients had increased frequencies of $TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ double negative (DN) T cells (83%), which are pathognomonic of autoimmune lymphoproliferative syndrome due to defects in the FAS pathway [83, 87, 88]. Due to the propensity of this cell type to have immunoregulatory, T_H-like effector, or cytotoxic functionalities, further characterization of DN T cells due to *STAT3* GOF variants is required to determine if and how these cells contribute to disease. Limited understanding of whether this population is expanded via extrinsic overactive immune signals or by an intrinsic STAT3-regulated mechanism currently limits connecting this population to disease pathogenesis and treatment options [89].

As STAT3 plays a crucial role in the development of T_{FH} and T_H17 cells, it naturally follows that overactive STAT3 may result in pathogenic over-enhancement of these populations. However, increased frequencies of these cells have only been observed in a minority of patients—for example, 27% of patients had an increase in IL-17-producing $CD4^{+}$ T cells [83]. This suggests that the generation of pathogenic T_H17 cells is not a default consequence of hyper-active STAT3 function, and thus not a general driver of autoimmunity in STAT3 GOF patients, despite the requirement for STAT3 in the generation of these effector CD4+ T cell subsets. Further research is required into possible T_{FH} defects in this patient cohort.

In contrast to T_{FH} and Th17 cells, $CD4^{+}CD25^{+}FOXP3^{+}$ T regulatory cells (Tregs) have been postulated to be reduced in number and/or function due to STAT3 overactivation, representing a viable mechanism of systemic autoimmunity contributing to the clinical presentation of autoimmune cytopenia [86]. Moreover, diminished expression of CD25, the high-affinity receptor of IL-2 and a canonical receptor expressed by Tregs, suggests a functional defect in STAT3 GOF patients due to a reduced ability to absorb pro-stimulatory IL-2 [54, 85, 90, 91]. Mechanistically, over-active STAT3 signalling has been shown to reduce activation of both STAT1 and STAT5 signalling axes [90, 91]. Notably, individuals with biallelic *STAT5B* variants develop a severe immune dysregulatory condition due to a Treg deficiency [25, 92]. Therefore, reduced IL-2/STAT5 signalling is likely causal for the reduction in T_{reg} number and function in STAT3 GOF [93]. However, Treg numbers are reduced in only ~40% of STAT3 GOF syndrome cases. Similarly, when a human pathogenic mutant is incorporated into a mouse model, the Treg compartment is largely intact; these observations suggest this cellular aberration is not a primary driver of clinical phenotype [83, 94]

In the B cell compartment, an expanded population of $CD21^{lo}Tbet^{+}CD19^{+}$ B cells has been routinely observed in STAT3 GOF patients [85, 95]. This population is often

enriched in chronic infection (human immunodeficiency virus, malaria infection), autoimmunity (systemic lupus erythematosus, rheumatoid arthritis), primary immunodeficiency (common variable immunodeficiency), and malignancy (diffuse large B cell lymphoma) implicating these cells in the pathogenesis of a broad range clinical outcomes [95]. Overall, STAT3 GOF syndrome represents an ideal monogenic human disease to study the development of adaptive immune cell types to unravel the contribution they make to homeostatic and pathogenic human immune responses.

Despite lymphoproliferative manifestations, it is fascinating that $CD4^+$ T cell and B cell lymphopenia occurs in ~50% and ~30–35% of patients, respectively [83]. Moreover, hypogammaglobulinemia—particularly IgG (51%) and IgA (36%)—presents consistently across the cohort. These cellular and humoral defects likely contribute to infectious susceptibilities in this disease [83]. Lymphopenia due to the over-activation of a pathway essential in the differentiation of human lymphocytes further highlights the need for balanced cytokine-mediated immune signalling to maintain immune homeostasis.

Interestingly, STAT3 GOF syndrome can present in some cases in infancy with Type 1 diabetes mellitus (T1DM). Studies on neonatal T1DM patients provide useful insight in dissecting genetic and molecular causes of self-reactive immunity. Warshauer *et al.* identified a germline *STAT3* GOF pathogenic mutation causing neonatal T1DM which also recapitulated diabetes in a mouse model. This study identified aberrant cytotoxic $CD8^+$ T cells which evade exhaustion and contain a TCR repertoire reactive to β-islet associated self-antigen, thus driving T1DM [96]. A recent study also performed in a *Stat3* GOF mouse model showed $CD8^+$ T cells mediating self-reactivity through upregulation of NKG2D, an activating receptor expressed by NK and $CD8^+$ T cells that recognizes stress-induced MHC-class I-like ligands [97], as well as NK and $CD8^+$ T cell effector molecules IFN-γ, granzymes, and perforin [97]. Therefore, it would be interesting to characterize in more detail the role of cytotoxic $CD8^+$ T cells and NK cells in human *STAT3* GOF, as this compartment potentially contributes to disease manifestations.

Despite all the work performed over the last decade into STAT3 GOF Syndrome, it is not surprising further effort is needed to understand the complexity and implications of such a heterogeneous set of molecular and cellular phenotypes observed in affected individuals.

STAT3 in malignancy

Primary immunodeficiency commonly results in an increased propensity of developing malignancy, thus highlighting the vital role the immune system plays in human tumour surveillance. This is indeed the case for STAT3 HIES with increased instances of malignancy (8.2% in STAT3 HIES vs 2.1% general population) and dramatically reduced median age of diagnosis (26.5 years in STAT3 HIES vs 67 years in the general population) [80]. Non-Hodgkin lymphoma developed in 5.1% of 158 STAT3 HIES patients surveyed in one study, making this the most common malignancy [80]. Mechanisms driving hematological malignancy in STAT3 HIES remain incompletely understood, however defective NK and $CD8^+$ T cell function (reduced expression of NKG2D) may decrease immune surveillance of malignant cells, and chronic inflammation may drive B cell transformation [80, 98].

Patients with germline *STAT3* GOF variants also have an increased incidence of malignancy. Cohort studies suggest ~11% of patients develop malignancy, most commonly haematological malignancy, emphasizing the crucial role STAT3 plays in this cellular compartment. Of particular note, somatic *STAT3* GOF variants are highly enriched in large granular lymphocytic leukemias (LGL) and inflammatory hepatocellular adenoma (IHCA) [99, 100]. About 70% of LGLs and 12% of IHCAs have been approximated to result from *STAT3* GOF driver variants revealing the potency of STAT3 as an oncogene. Resistance to Fas-mediated apoptosis has been suggested as a mechanism for oncogenicity in LGL [101]. The correlation between the concurrence of malignancy and self-reactive autoimmunity through *STAT3* GOF variants is a growing paradigm, suggesting disease progression develops through similar molecular mechanisms [97, 99, 102].

Discussion

Increased accessibility of next-generation sequencing to identify candidate inborn errors of immunity has reinforced the importance of candidate variant validation. STAT3 HIES and STAT3 GOF syndrome clearly have heterogeneous disease presentations. Therefore, rigorous testing of novel *STAT3* variants is essential in confirming the impact of monogenic perturbations on biochemical, cellular, and physiological functions. Interestingly, a mutation in the same amino acid residue can result in either STAT3 GOF (N647I, K658N) or STAT3 LOF (N647D, K658E). This region in the SH2 domain directs docking of STAT3 to cytokine receptors and subsequent STAT3 dimerization. Thus, the nature of protein–protein interactions mediated by this region of the SH2 domain is likely to determine whether loss or gain-of-function occurs, thereby resulting in disease. Observing LOF and GOF at the same residues also identifies critical residues at the interface of key molecular interactions, be it protein to protein or protein to consensus DNA and directly contributes to the affinity of such interactions. In-depth molecular investigation of these residues is important so as to be able to potentially manipulate these interactions therapeutically to treat STAT3 HIES or STAT3 GOF. Similarly, a previously reported *STAT3* LOF mutation has recently been re-defined as having both LOF and GOF properties [103]. This rare phenomenon is a timely reminder of the complexity of genetic diseases, the importance of thorough candidate variant assessment, and to stray away from the binary notion of LOF and GOF dualism. Rare but poignant reports suggesting 'LOF' variants with 'GOF' properties and vice-versa support this notion [66, 86]. It is entirely conceivable that disparate *STAT3* variants soon to be identified will elicit differing signalling alterations by cell type or in response to different cytokine stimuli. Thus, evaluating biochemical, cellular, and clinical disease progression per mutation and per patient is critical in determining the type of STAT3-related disease, and may illuminate effective treatment regimens or therapeutic targets by improving patient outcomes.

This has become more relevant with evidence that JAK/STAT1 inhibition via ruxolitinib can successfully improve outcomes in STAT3 HIES patients [104]. This ingenious treatment regime was only considered by investigating molecular mechanisms explaining shared clinical features resulting from *STAT1* GOF and *STAT3* LOF variants. This raises the

question of whether a similar approach can be effective in STAT3 GOF due to its overlapping disease pathogenesis with STAT5 LOF [105].

To date, careful dissection of pathogenic *STAT3* LOF and GOF variants has provided a wealth of information regarding the non-redundant functions of STAT3 in human immune mechanisms. This has provided considerable benefit for not only patients carrying these variants but also patients suffering from diseases impacting related pathways. However, two key phenomena in STAT3-associated immunological biology warrant further investigation. Understanding the mechanism driving hyper-serum IgE levels in patients carrying *STAT3* LOF variants could provide considerable insight into B cell biology in the context of allergic responses. Little is known regarding crucial genes mediating IgE-driven allergic responses despite such a high disease burden across populations. Further, the paradox of simultaneous hypogammaglobulinemia due to both pathogenic *STAT3* LOF and *STAT3* GOF variants and how this can educate us on vaccine-induced immunity remains a crucial question. Such investigation will strengthen future vaccine design for not only these rare cohorts but also the broader population. As we continue to be inundated with 'big data' provided by the next-generation sequencing revolution, it is pertinent to appreciate intricacies garnered through careful investigation of novel pathogenic variants, as has been strikingly evident through STAT3-associated disease. Notwithstanding the breadth of what has already been discovered, there is still a long way to go in understanding a gene so ubiquitous in human biology.

The animal research adheres to the ARRIVE guidelines

Not applicable.

Data availability statement

Not applicable.

Competing interests

The authors have no competing conflicts of interest to declare.

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Author contributions

JM prepared the initial draft of this manuscript; AG, CSM, and SGT edited subsequent drafts; SGT prepared and submitted the final draft.

Clinical trial registration

Not applicable.

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