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STAT3 is a central regulator of lymphocyte differentiation and function

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Signalling in lymphocytes through cytokine receptors is critical for their development, activation and differentiation into effector cells that mediate protection against pathogens and provide the host with protective immunological memory. The essential role of cytokine signalling has been established not only by the generation and examination of gene-targeted mice, but also 'Experiments of Nature' whereby monogenic mutations cause primary immunodeficient conditions characterised by impaired immunity to infectious diseases due to compromised lymphocyte function. Mutations in *STAT3* cause autosomal dominant hyper-IgE syndrome. Here, we will review how the study of STAT3-deficient individuals has revealed non-redundant functions of STAT3 and specific cytokines in human lymphocyte biology, and have delineated mechanisms underlying the distinct clinical features of autosomal dominant hyper-IgE syndrome.

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Introduction

The generation of lymphocytes from hematopoietic precursors, and their subsequent differentiation into effector cells, requires signals provided by surface receptors including those that bind Ag, co-stimulatory and adhesion molecules, and cytokines. The biological effects of cytokines are largely mediated by JAK/STAT signalling pathways activated downstream of their specific receptors [1–3]. Four mammalian JAKs (JAK1, JAK2, JAK3, Tyk2) and seven STATs (STAT1, 2, 3, 4, 5a, 5b, 6) have been identified [1,2]. JAKs associate with the cytoplasmic domains of multimeric cytokine receptors and, following engagement by specific ligands, phosphorylate cytoplasmic tyrosine residues which act as docking sites for STATs. Receptor-associated STATs undergo JAK-mediated phosphorylation, which promotes formation of dimers that translocate to the nucleus and bind specific DNA sequences, promoting transcription of target genes and regulating the epigenetic state of chromatin [1–3].

Cytokines are fundamental for lymphocyte development and differentiation. This is evidenced by the immunodeficient and autoimmune states that arise from monogenic mutations in genes encoding cytokines (*IL17F*, *IL10*, *IL12B*), their receptors (*IL2RG*, *IL7RA*, *IL12RB1*, *IFNGR1/2*, *CD25/IL2RA*, *IL10R*, *IL21R*), or components of downstream signalling pathways (*JAK3*, *TYK2*, *STAT1*, *STAT2*, *STAT3*, *STAT5A*) (see Table 1 for summaries) [3–6]. Here, we review the function of STAT3 in generating efficient cellular and humoral immune responses, as revealed from analysis of STAT3-deficient humans and mice.

Autosomal dominant hyper-IgE syndrome (AD-HIES): human STAT3 deficiency

In 1966, Davis and colleagues reported a new immunodeficiency in two unrelated girls with severe eczema and staphylococcal lung and skin infections [7]. Remarkably the skin infections were often abscesses that lacked significant inflammation (erythema and warmth) of the effected tissues. In 1972, Buckley reported two boys with similar presentations but emphasised their coarse facial features, chronic dermatitis, pneumatocoeles and extreme hyper-IgE. These patients exhibited poor T-dependent (TD) Ag-specific Ab responses, despite normal levels of serum IgM, IgG and IgA. The children had uncomplicated infections with measles, but did have severe mucocutaneous fungal infections [8]. This condition was termed autosomal dominant hyper-IgE syndrome (AD-HIES; see Figure 1) [9,10].

These initial descriptions provide accurate accounts of the clinical phenotype of this rare disease; infection of the skin, lungs and upper airways with *S. aureus*, together with recurrent pneumonia and mucocutaneous — but not

Table 1

Gene mutations in cytokine signalling pathways that cause human immunodeficiency

Mutated gene	Mechanism of disease	Clinical features of disease and Infectious susceptibility	Cytokines involved
IL2RG	X-linked loss of function	• $B^+T^-NK^-$ X-linked SCID	• IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
JAK3	Autosomal recessive (AR) loss of function	• $B^+T^-NK^-$ AR SCID	• IL-2, IL-4, IL-7, IL-9, IL-15, IL-21
IL7RA	AR loss of function	• $B^+T^-NK^+$ combined immunodeficiency	• IL-7, TSLP
TYK2	AR loss of function	• Originally considered as AR hyper-IgE syndrome, but not all patients have elevated IgE levels or chronic mucocutaneous candidiasis (CMC) • Mendelian susceptibility to mycobacterial disease (MSMD), especially disseminated BCG • Also susceptible to infection with salmonella, brucella and staphylococci • Recurrent cutaneous herpes simplex infection, VZV reactivation	• Type I IFNs • IL-6, IL-10 families • IL-12, IL-23
STAT1	AR complete loss of function	• MSMD (usually BCG) • Infection with herpes viruses (often fatal)	• Type I and II IFNs • IL-6, IL-10, IL-21, IL-27
	Autosomal dominant (AD) or AR partial loss of function AD gain of function	• MSMD (Disseminated BCG and non-tuberculous mycobacterial [NTM] infections) • CMC • Susceptibility to infection with other pathogens including viruses (varicella, HSV, EBV), dimorphic yeast (histoplasmosis, coccidioidomycosis) and non-tuberculous mycobacteria • Autoimmune manifestations including endocrinopathy (IPEX-like disease) • Vascular anomalies • Defects in humoral immunity • Defects in NK cell function	
IL12RB1	AR loss of function	• MSMD (BCG, NTM or *M. tuberculosis*) • Non-typhoidal salmonella infection, recurrent leishmaniasis • Some patients are also affected by other intracellular pathogens; dimorphic fungi (paracoccidioides and coccidioidomycosis) and candida infections	• IL-12, IL-23
IFNGR1		• MSMD • Defect in granuloma formation in the setting of NTM depends on the severity of the IFNγ signalling defect	• Type I and II IFNs
	AR complete loss of function	• Severe infections, plus increased susceptibility to CMV, VZV, *L. monocytogenes*	
	AR partial loss of function AD partial (dominant negative)	• Presents later in life with rather limited disease • Most common form among IFNGR defects, and most favourable treatment response profile • Infections are localised or disseminated BCG or NTM, histoplasmosis, or salmonellosis, and usually occur later in childhood or adolescence • Multifocal mycobacterial osteomyelitis is the hallmark	
IFNGR2	AR complete loss of function AR partial loss of function AD partial (dominant negative)	• Severe infections • Presents later in life with rather limited disease • Very rare — CMV, NTM infections	• Type I and II IFNs
STAT2	AR loss of function	• Susceptible to vaccine-strain measles; otherwise healthy	• Type I IFNs

Table 1 (*Continued*)

Mutated gene	Mechanism of disease	Clinical features of disease and Infectious susceptibility	Cytokines involved
STAT3	AD loss of function	• AD hyper-IgE syndrome (AD-HIES) • Susceptibility to infections with *S. aureus*, *S. pneumonia*, *C. albicans* • EBV viraemia and VZV reactivation • Eczema, vascular, musculoskeletal, dental and connective tissue defects • B-cell lymphoma	IL-6, IL-10 and IFN families, IL-21, IL-23, plus others
STAT5b	AR loss of function	• Growth hormone insensitivity • Tregs very diminished in number and dysfunctional, leading to immune dysregulation and autoimmunity, lymphocytic infiltrates in the lungs (lymphocytic interstitial pneumonitis), skin, T- and NK-cell lymphopenia, immunodeficiency with susceptibility to bacterial, viral (VZV), and fungal infections	• IL-2, IL-15, TSLP • Growth hormone
IL21R	AR loss of function	• Combined immunodeficiency • Sinopulmonary infections and bronchiectasis • Disseminated cryptosporidium infection • Susceptibility to infection with *Pneumocystis jirovecii* • Impaired humoral immunity • Increased serum IgE	• IL-21
IL17RA; *IL17F*	AR deficiency of IL-17RA; partial deficiency of IL-17F homo- and heterodimers (with IL-17A)	• CMC	• IL-17A • IL-17F
CD25	AR loss of function	• IPEX-like disease lymphocytic infiltration of skin, lung, liver, gut, and bone and endocrinopathy • Chronic CMV infection, susceptibility to bacterial and fungal infections	• IL-2
IL10; *IL10RA*/ *IL10RB*	AR loss of function	• Early onset fistulising inflammatory bowel disease	• IL-10 (also IL-22, IL-26 and IFNλ for *IL-10RB* mutations)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; BCG, *Bacillus Calmette–Guérin*; CMC, chronic mucocutaneous candidiasis; CMV, cytomegalovirus; EBV, Epstein Barr virus; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked; MSMD, Mendelian susceptibility to mycobacterial disease, SCID, severe combined immune deficiency; TSLP, thymic stromal lymphopoietin; VZV, varicella zoster virus. For more details, see Refs [4–6,26].

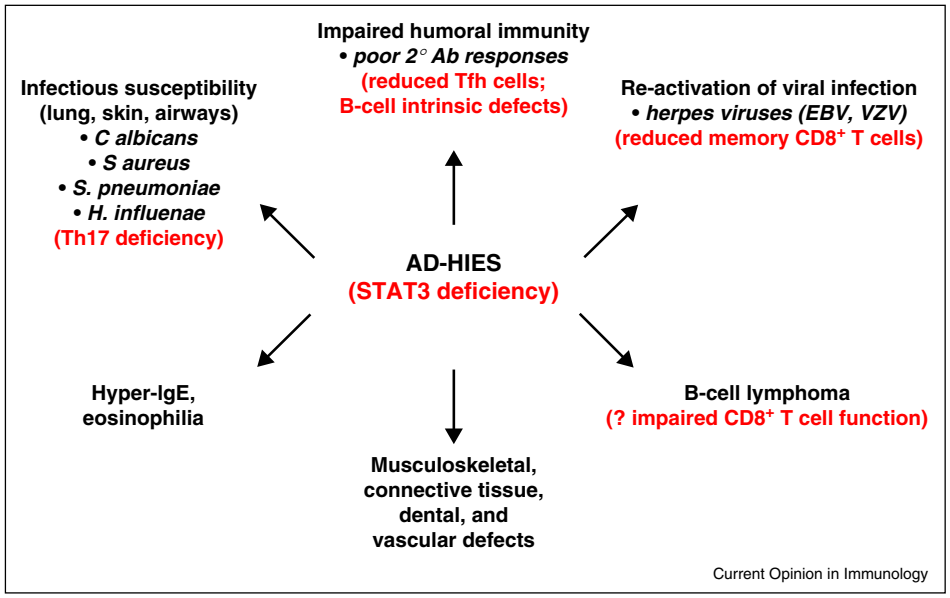
invasive systemic — candidiasis [9,10]. Strikingly, viral infections are not a distinguishing aspect of AD-HIES, although there may be impaired control of herpes viruses, leading to reactivation of EBV and VZV infection [11••,12•]. Eosinophilia is usually present, while a small proportion of patients develop B-cell lymphoma [9,10,11••]. Interestingly dental, musculoskeletal, connective tissue and vascular features manifesting as retention of primary teeth, scoliosis, osteopenia, frequent fractures, short stature, joint hyperextensibility as well as intracranial and coronary aneurysms represent the non-immunological features of AD-HIES ([9,10]; Table 1 and Figure 1).

In 2007, heterozygous mutations in *STAT3* were identified as the genetic etiology of AD-HIES [13••,14••]. This discovery underscored the importance of STAT3 in regulating critical aspects of cellular and humoral immunity. Constitutive deletion of *Stat3* in mice is embryonically lethal due to a requirement for STAT3 in trophoblast invasion and placental development [15]. In contrast, the dominant negative *STAT3* alleles in AD-HIES patients reduce, but do not abolish, STAT3 function [14••]. Thus, while such mutations are not lethal, they nonetheless severely compromise immunity.

STAT3 regulates CD4$^+$ T cell differentiation.

Naïve CD4$^+$ T cells can differentiate into numerous effector subsets — Th1, Th2, Th9, Th17, Th22, T follicular helper (Tfh), regulatory T (Treg) cells — with unique roles in protective immunity against infectious diseases, as well as autoimmune or inflammatory disorders (Figure 2). This process is mediated by cytokines present in the microenvironment, and the signalling cascades they activate ([16–18]; Figure 2). Studies of AD-HIES patients and conditional STAT3-deleted mice have revealed multiple roles for STAT3 in generating several CD4$^+$ T cell subsets.

Figure 1



Clinical features and cellular defects in autosomal dominant hyper-IgE syndrome (AD-HIES) due to mutations in *STAT3*. The key clinical features of AD-HIES, and possible defects in B cells and T cells that underlie these features, are indicated. The cause of hyper-IgE, as well as the non-immunological defects, remains to be determined.

Th17 cells

Th17 cells can be induced *in vitro* from naïve precursors following stimulation with TGF-β and IL-6; the Th17 program is imprinted by additional cytokines such as IL-23 [17]. IL-6 and IL-23 activate STAT3 providing the basis for the initial discovery in mice that STAT3 plays a critical role in generating Th17 cells [19–22]. Analysis of STAT3-deficient patients revealed a lack of Th17-type cells *ex vivo* [6,23–25], as well as in response to polarising conditions *in vitro* [23] confirming the role of STAT3 in Th17 differentiation in humans as well (Figure 2). The role of IL-23 *in vivo* in this process was evident from patients with mutations in *IL12RB1* — a component of the IL-23 receptor — who also had reduced Th17 cells ([24]; Table 1). However, the reduction in Th17 cells in IL-12RB1-deficiency was not as severe as in STAT3-deficiency, supporting a role for additional cytokines (e.g. IL-6) in Th17 generation [24]. As Th17 cells, and particularly IL-17 cytokines, are requisite for protection against fungal infections [26], the Th17 defect in AD-HIES explains the high incidence and persistence of mucocutaneous candidiasis in these individuals (Figure 1). Interestingly, IL-21R-deficient patients do not develop candidiasis and have intact Th17 cells ([27••]; Table 1), implying that — despite suggestions to the contrary from studies in mice [17,28] — IL-21 does not play a significant role in the generation and/or maintenance of human Th17 cells.

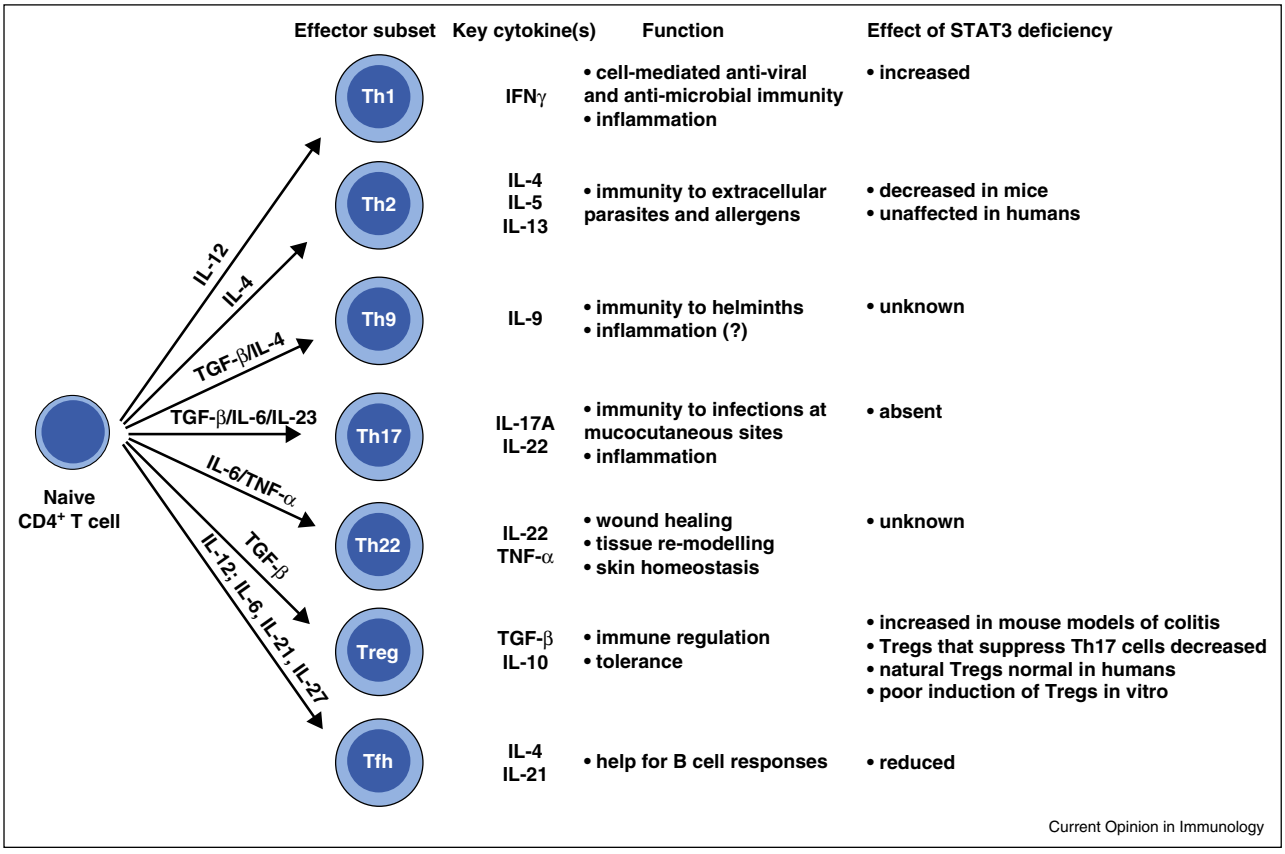
STAT3 controls Th17 differentiation by inducing genes that characterise Th17 cells, for example, transcription factors RORγt and RORα, and effector cytokines IL-17A, IL-17F and IL-21, which can be further induced by RORγt [20–22,28–32]. Interestingly, STAT3 may also promote Th17 cell generation by repressing *Tbx21* (T-bet) [21], *Gata3* [33•] and *Foxp3* [21], thus impeding the differentiation of naïve $CD4^+$ T cells to Th1, Th2 or Treg fates, respectively. Indeed, STAT3-deficient human $CD4^+$ T cells generate more $IFN\gamma^+$ Th1-type cells *in vitro* than do normal $CD4^+$ T cells ([34••]; Figure 2).

Treg cells

In mice, STAT3 can either promote or restrain Treg formation by differentially regulating FoxP3 expression (Figure 2). For instance, IL-6/STAT3 signalling inhibits Foxp3 expression; thus few Tregs are generated *in vitro* under Th17 (TGF-β/IL-6) conditions [20–22]. Interestingly, IL-27 also represses TGF-β-induced Foxp3 expression by activating STAT3, which directly silences *Foxp3* [35]. Consistent with this, in a murine model of colitis, STAT3-deficient $CD4^+$ T cells exhibited impaired Th17 generation, but yielded increased Tregs [30]. Conversely, STAT3 may also act in Tregs to generate a subset that specifically suppresses the function of Th17 cells as deletion of STAT3 in Tregs resulted in increased Th17-associated cytokines and pathology, without affecting Th1 or Th2 cytokines [36]. However, the cytokines that act via STAT3 to generate Tregs that control Th17 responses are unknown.

Despite these studies in mice, it is unclear whether STAT3 has an intrinsic role in the differentiation of

Figure 2



CD4$^+$ T cell differentiation — effects of STAT3 deficiency. Naïve CD4$^+$ T cells can differentiate into numerous subsets of effector cells with distinct roles in protection against pathogen infection. CD4$^+$ T cell differentiation is regulated by cytokines provided within the stimulatory microenvironment; this requires cytokine-mediated activation of various transcription factors, including STATs, which in turn induce expression of key cytokines that underlie the effector function of each subset of CD4$^+$ T cells (modified from [16]). The consequence of STAT3 deficiency on the generation of each subset in mice and humans (where known) is indicated.

human Tregs, as these cells are generated at normal frequencies in AD-HIES, and exhibit normal suppressive function *in vitro* (Figure 2; [23,37•]). However, DCs from STAT3-deficient patients are unable to respond to IL-10, impairing their ability to become tolerogenic and induce Tregs from naïve CD4$^+$ T cells [37•]. Interestingly STAT3-deficient CD4$^+$ T cells produce less IL-10 than normal CD4$^+$ T cells [23]. Thus, although Tregs are generated normally in STAT3-deficient patients, the inability of STAT3-deficient DCs to convert naïve CD4$^+$ T cells into Tregs, together with poor IL-10 production, opens the possibility of defective immune regulation, which could potentially contribute to atopy in AD-HIES.

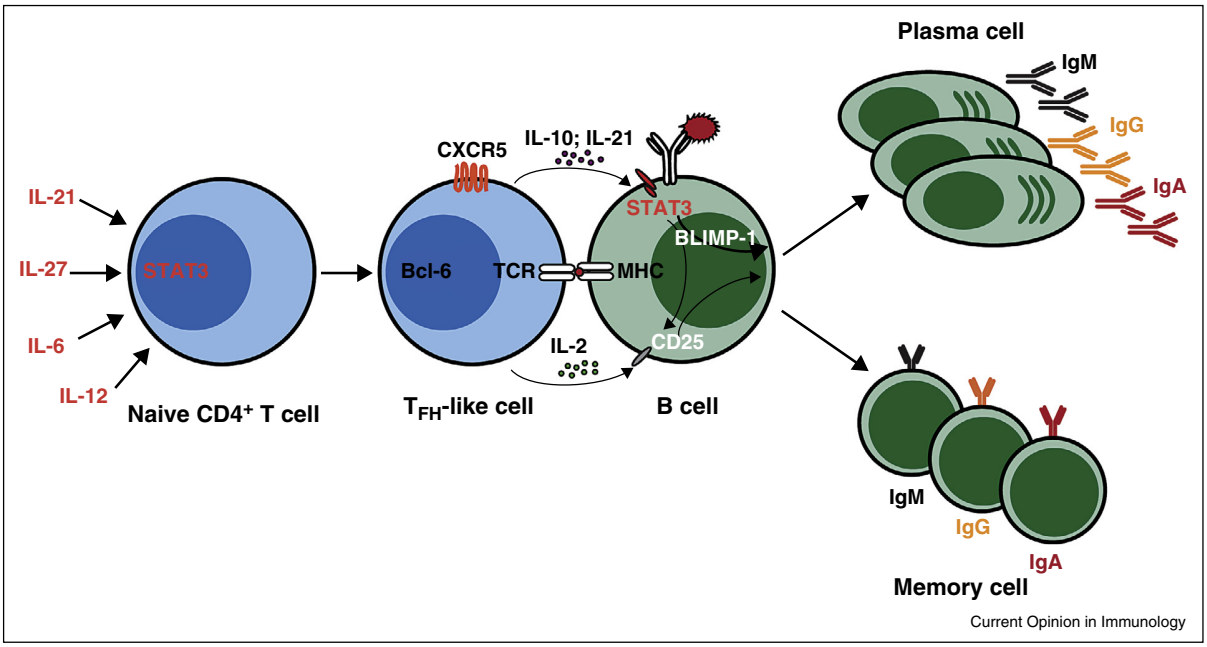
Th2 cells

Dysregulated IgE production in AD-HIES is suggestive of excessive Th2 function. However, the differentiation of naïve human CD4$^+$ T cells into Th2-type cells *in vivo* and *in vitro* appears to be unaffected by *STAT3* mutations [6,23,37•]. Despite these findings, STAT3 deficiency in murine CD4$^+$ T cells compromised their ability to produce the signature Th2 cytokines IL-4, IL-5 and IL-13, and express *Gata3*, under Th2 polarising culture conditions *in vitro* (Figure 2; [33•]). These defects are likely due to direct regulation of Th2 transcription programs by STAT3, which was found to bind directly to *Gata3*, *cmaf* and *Batf* [30,33•]. Interestingly, STAT3-deficiency reduced STAT6 binding to these target genes, abolished STAT6-dependent Th2 induction *in vitro*, and ameliorated disease in a murine model of allergic inflammation [33•]. Thus, STAT3, together with STAT6, is a key regulator of Th2 differentiation. But how STAT3 regulates the function of STAT6, as well as the relative role of STAT3 in human Th2 responses, remains unknown.

Tfh cells

Tfh cells provide help to B cells to differentiate into memory and plasma cells, thereby establishing long-lived

Figure 3



B-cell and T-cell intrinsic functions of STAT3 underlie successful humoral immune responses. $CD4^+$ T cells differentiate into Tfh cells under the influence of cytokines including IL-6, IL-12, IL-21 and IL-27. The provision of IL-21 by Tfh cells allows for the differentiation of activated B cells into memory B cells and plasma cells. This is achieved by the induction of Blimp-1 and CD25, which promote the formation of plasma cells. The exact mechanism by which IL-21 promotes memory cell formation is unknown. Other cytokines, such as IL-10, also promote Tfh-mediated B-cell differentiation. This pathway of Tfh-dependent B-cell differentiation is compromised by STAT3-deficiency at several stages. First, fewer Tfh cells are generated in patients with *STAT3* mutations, thus limiting B-cell help. Second, *STAT3* mutations abolish the ability of naïve B cells to respond to the stimulatory effects of IL-21 and IL-10, thereby preventing these cytokines from inducing the formation of plasma cells from naïve precursors.

humoral immunity [38]. Tfh cells are typically detected in secondary lymphoid tissues, however a circulating counterpart of Tfh cells can be detected in peripheral blood of humans and mice [38]. AD-HIES patients have a reduction in circulating Tfh cells [34••,39,40••], and STAT3-deficient $CD4^+$ T cells failed to differentiate *in vitro* into IL-21-producing Tfh-like cells (Figures 2 and 3; [34••,41]). These findings confirm studies in mice demonstrating that STAT3-deficiency impedes Tfh formation *in vivo* by compromising signalling downstream of IL-6, IL-21 and/or IL-27 (Figures 2 and 3; [38,41–43]). However, STAT1 may compensate for STAT3 in some instances [43]. This deficit in Tfh cells would contribute to the inability of STAT3-deficient patients to generate normal titres of Ag-specific Ab ([8,44–46,47•,48•]; Figures 1 and 3).

STAT3 and B cell function

STAT3 regulates the commitment of Ag-specific B cells to the pool of long-lived plasma cells

Although B cell development and production of basal serum IgM, IgG and IgA are normal in AD-HIES and mice that lack STAT3 specifically in B cells, STAT3-deficiency causes defective TD Ag-specific Ab responses [8,44–46,47•,48•,49]. While STAT3-deficient mice only have a reduction in IgG1-secreting plasma cells [49], AD-HIES patients have defects in both IgG and IgA Ag-specific responses [8,44–46,47•,48•]. STAT3-deficient humans also lacked memory B cells [11••,40••,47•,48•], which was not replicated in mice with STAT3-deficient B cells [49]. There are several possible explanations for these differences. First, they may result from species-specific differences in the regulation of B cell differentiation. Second, as memory formation in mice was assessed only at a single time point post-immunisation, the paucity of memory B cells in STAT3-deficient humans may stem from a defect in long-term survival rather than their generation. Third, the differences may reflect B cell-extrinsic actions of STAT3, since in mice only B cells lacked STAT3, whereas in humans all cells are STAT3-deficient. Defects in Tfh formation by STAT3-deficient $CD4^+$ T cells provide one possible example ([34••,39,40••], Figures 2 and 3). However, there is clearly an intrinsic defect in STAT3-deficient naïve B cells, as they are unable to differentiate into plasmablasts *in vitro* in response to IL-10 and IL-21 (Figure 3; [40••,47•]).

Although analysis of AD-HIES patients revealed a requirement for STAT3 in generating Ag-specific memory and plasma cells, it did not identify the STAT3-activating

cytokine(s) involved in this process *in vivo*. The recent discovery of patients with *IL21R* mutations and the observation that they lack memory B cells and have defects in humoral immunity ([27••,40••]; Table 1) established that IL-21 is likely to be the predominant cytokine upstream of STAT3 that regulates human B cell function (Figure 3). This is consistent with its superior ability to induce *PRDM1* (Blimp-1) and *XBP1* in naïve B cells, and mediate their differentiation into plasmablasts *in vitro*, compared to other STAT3 cytokines, IL-6 and IL-10 [40••,47•,50,51]. IL-21 also promotes Ab production by inducing CD25 on activated human B cells in a STAT3-dependent manner, thereby priming them to respond to IL-2 (Figure 3; [52]). Thus, IL-21R/STAT3 signalling is central to generating TD humoral immune responses in humans, and defects in this pathway underlie poor serological memory in AD-HIES and IL-21R-deficiency (Figures 1 and 3). Remarkably, the small population of memory B cells that is generated in AD-HIES is functionally normal with respect to responding to STAT3 cytokines and yielding plasmablasts [40••]. This highlights differences in requirements for activating naïve and memory B cells, and suggests that STAT3-deficient memory B cells may contribute to variable levels of Ag-specific Ab titres detected in some AD-HIES patients [47•,48•].

CD8$^+$ T cells

STAT3 deficiency also affects CD8$^+$ T cells in humans and mice. STAT3-deficient human CD8$^+$ T cells are impaired in their ability to upregulate expression of perforin and granzyme B in response to IL-21 and IL-15, however this can be circumvented by TCR ligation [53•]. Additionally, mice whose CD8$^+$ T cells lack STAT3 have no defect in early viral clearance [54•]. These findings suggest sufficient redundancy exists in STAT3-deficient CD8$^+$ T cells to ensure acquisition of cytolytic function in response to pathogen infection, despite impaired signalling to some key cytokines. Despite this apparently intact effector function, STAT3-deficiency did impact the generation of memory CD8$^+$ T cells in both species [12•,53•,54•], possibly due to reduced expression of the transcription factors Bcl-6 and eomesodermin, which govern the formation of some memory cell subsets [12•,54•]. Murine studies found that combined deficiency of IL-10 and IL-21 phenocopied the memory defect in STAT3-deficient CD8$^+$ T cells [54•]. IL-21R-deficient humans also had fewer memory CD8$^+$ T cells, suggesting that in humans IL-21 — via STAT3 — contributes to the formation and/or maintenance of CD8$^+$ T cell memory [53•]. STAT3-deficient CD8$^+$ memory T cells failed to protect mice from secondary viral infections [54•], while expression of IL-21R on murine CD8$^+$ T cells is required to control chronic viral infection [55]. Interestingly, AD-HIES patients were found to have impaired control of reactivation of chronic infection by EBV and varicella [11••,12•]. Thus, it is possible that defective CD8$^+$ T cell differentiation and function induced by IL-21 underlies poor immunity in STAT3-deficient humans to latent herpes viruses (Figure 1). However, as IL-21R-deficient individuals are not adversely affected by herpes viruses, additional STAT3-activating cytokines are also likely to be involved in this response. Given the role that CD8$^+$ T cells play in anti-tumour immunity, it is also possible that defective cytotoxicity by these cells contributes to lymphoma development in AD-HIES ([9,10,11••]; Figure 1).

Conclusions

The discovery of molecular lesions that cause primary immunodeficiencies provide the opportunity to identify specific functions of genes in different cell lineages, thereby defining the pathogenesis of these diseases. This is indeed the case for AD-HIES, with defined defects in CD4$^+$ T cells, B cells and CD8$^+$ T cells due to STAT3 deficiency potentially contributing to infection with specific pathogens, defective humoral immunity and susceptibility to lymphoma, respectively, in affected individuals. However, many issues remain unaddressed, such as the cause of hyper-IgE and the non-immune features of AD-HIES, as well as the impact of *STAT3* mutations on other human hematopoietic cell lineages such as NK cells and semi-invariant T cells, granulocytes and myeloid cells. This is particularly relevant as IL-6 and IL-23 can induce RORγt and IL-17 in human and murine γδ T cells [56–58] and neutrophils [59], which plays important roles in host defence against fungal and bacterial infections that are common in AD-HIES [9,10]. Furthermore, as there are some clinical commonalities between *STAT3* loss-of function and *STAT1* gain-of function mutations (Table 1, Refs [5,6]), it is clear that the net outcome of lymphocyte activation is not simply the result of linear signalling through one predominant STAT but rather complex interactions between numerous STATs that not only complement but also antagonise each others function. Ongoing detailed molecular, cellular and biochemical analyses of these patients will hopefully elucidate these interactions further and yield improved therapies for these disorders.

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