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STAT3 signaling in immunity

Emily J. Hillmer^{1,*}, Huiyuan Zhang^{1,*}, Haiyan S. Li^{1,*}, and Stephanie S. Watowich^{1,2}

¹Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

²The University of Texas Graduate School of Biomedical Sciences, Houston, TX 77030

Abstract

The transcriptional regulator STAT3 has key roles in vertebrate development and mature tissue function including control of inflammation and immunity. Mutations in human *STAT3* associate with diseases such as immunodeficiency autoimmunity and cancer. Strikingly, however, either hyperactivation or inactivation of STAT3 results in human disease, indicating tightly regulated STAT3 function is central to health. Here, we attempt to summarize information on the numerous and distinct biological actions of STAT3, and highlight recent discoveries, with a specific focus on STAT3 function in the immune and hematopoietic systems. Our goal is to spur investigation on mechanisms by which aberrant STAT3 function drives human disease and novel approaches that might be used to modulate disease outcome.

Graphical Abstract

Keywords

STAT3; cytokines; immune system; inflammation

Correspondence: Stephanie S. Watowich, PhD, Department of Immunology, MD Anderson Cancer Center, P.O. Box 301402, Houston, TX 77030, USA. swatowic@mdanderson.org, Phone (713)563-3262, FAX(713) 563-3280.
*denotes equal contribution

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Introduction: STAT3 discovery, structure and transcriptional function

STAT3 was discovered over 20 years ago as a component of the interleukin-6- (IL-6) activated acute phase response factor (APRF) complex [1–3], which has a crucial role in stimulating expression of innate immune mediators in liver. This discovery led to rapid identification of STAT3 as a member of the STAT (Signal Transducers and Activators of Transcription) family, based on size similarity, antigenic and structural relatedness, as well as comparable DNA binding activity, to the interferon (IFN)-responsive STAT proteins. Subsequent work identified 7 members of the STAT protein family in mammals [3–6]. Activation of STAT3 is elicited by numerous cytokines and growth factors, including cytokines utilizing the IL-6 signal-transducing receptor chain gp130 (e.g., IL-6, oncostatin M, interleukin-11) or homodimeric cytokine receptors (e.g., granulocyte colony-stimulating factor, G-CSF), as well as growth factors that act through protein tyrosine kinase receptors (e.g., epidermal growth factor) [2, 3, 7, 8]. Moreover, STAT3 mediates important signal transduction cascades elicited by intracellular proteins such as activated Ras or tyrosine kinase oncoproteins (e.g., Src) [9–14]. Many early studies foreshadowed the multiple and distinct biological roles for STAT3 that are appreciated today. Accordingly, interest in STAT3 has risen substantially since its discovery, as judged by a survey of STAT3-immune system-related publications (Figure 1).

The primary amino acid sequence of STAT3 revealed a conserved Src homology 2 (SH2) domain and a C-terminal tyrosine residue (Y705 in mice) that becomes phosphorylated by Jak kinases upon cytokine stimulation, protein tyrosine kinase receptor signaling or intracellular protein tyrosine kinase activation [5, 9]. STAT3 forms homodimers by reciprocal SH2 domain-phosphotyrosine interactions between 2 monomers; this was identified as a key activating mechanism leading to stimulation of STAT3 transcriptional function. STAT3 also undergoes serine phosphorylation at position 727 (S727), a modification that enhances transcriptional activity [15–18]. STAT3 DNA association is mediated by a central DNA-binding region (Figure 2), while protein:protein association domains located at the STAT3 N- and C-terminal regions are also involved in transcriptional regulation. Numerous approaches including sequence comparisons, mutational analyses, biochemical and structural studies of STAT3 and other family members led to these important discoveries [19–31]. Further posttranslational modifications such as acetylation and methylation have been implicated more recently in STAT3 transcriptional function [32–35]. Moreover, STAT3 can be activated constitutively by engineered introduction of cysteine residues, which drive cytokine-independent dimerization, rendering oncogenic activity [36]. Several excellent reviews summarize the discovery of STATs and the intense work to characterize their signaling mechanisms and functions in the early days of the field [37–42].

More recently, unphosphorylated STAT3 (uSTAT3) has been recognized as an important transcriptional regulator [43–45]. In the unphosphorylated state, uSTAT3 binds similar DNA sites as tyrosine-phosphorylated and dimerized STAT3 (e.g., IFN- γ -activated sequence (GAS) elements), yet uSTAT3 works in collaboration with transcriptional regulators such as NF- κ B to control a cadre of genes not normally affected by tyrosine-phosphorylated STAT3 [34, 44, 46]. STAT3 also induces its own gene expression via a STAT3- *Stat3* positive autoregulatory loop [47]. Thus, STAT3 homodimers activated by cytokine or growth factor

receptors, as well as intracellular protein tyrosine kinases, have potential to boost total STAT3 protein amounts, increasing availability of uSTAT3 and STAT3 as a tyrosine kinase substrate. Accordingly, STAT3 autoregulation provides a mechanism to shape gene expression temporally throughout the duration of a cytokine response. Further work is needed to understand the global transcriptional impact of uSTAT3 and the tyrosine-phosphorylated form in the immune response as well as induction or maintenance of immunological diseases and cancer.

Non-transcriptional activities of STAT3

STAT3 also has important activities in cellular respiration, metabolism, autophagy and cancer that are separate from transcriptional roles mediated by the tyrosine-phosphorylated or unphosphorylated isoforms. In 2009, the intriguing discovery was made that STAT3 associates with mitochondria and therein regulates electron transport chain function as well as glycolytic and oxidative phosphorylation [13, 48]. Mitochondrial STAT3 is also implicated in control of the gamma-glutamyl cycle, production of glutathione and regulation of reactive oxygen species (ROS) [49]. Furthermore, in embryonic stem (ES) cells, STAT3 controls mitochondrial gene expression and respiration, mechanisms that optimize ES cell proliferation and maintenance [50]. By contrast, genetic screens identified STAT3 as a negative regulator of autophagy via inhibition of type III phosphoinositide 3-kinase (PI3K) or association with the eukaryotic translation initiation factor 2a (eIF2a) kinase 2/protein kinase R (EIF2AK2/PKR), respectively [51–53].

Significantly, the mitochondrial function of STAT3 is critical for Ras-mediated oncogenic transformation [13,14]. Ras proteins (e.g., KRas, NRas and HRas) belong to a family of small GTPases; gain-of-function (GOF) mutations in Ras homologs were the first oncogenic mutations identified in human cancers and are associated with a variety of malignancies [54–56]. Ras GOF mutations stimulate MEK-Erk signaling and STAT3 S727 phosphorylation, which is essential for Ras-mediated malignant transformation [14]. Recent studies using KRas-driven pancreatic and myeloproliferative tumor models demonstrated a requirement for STAT3 in oncogenesis in vivo, yet conditional *Stat3* ablation concomitant with KRas activation in lung epithelial cells had the unexpected result of enhancing Kras-driven lung tumor progression [57–59]. These disparate findings suggest additional complexity in the oncogenic activity of mitochondrial-associated STAT3, which may relate to tissue-specific roles. Thus, further studies using conditional *Stat3* ablation in spontaneous Ras-driven tumor models as well as investigation of mitochondrial STAT3 function in human tumor specimens are needed to clarify the function of mitochondrial STAT3 in cancer.

STAT3 mutations in human immune disorders

STAT3 inactivation and HIES

Autosomal dominant *STAT3* inactivating mutations in the human immunodeficiency condition termed Hyper immunoglobulin E syndrome (HIES) reveal a causal role for STAT3 loss-of-function (LOF) in human immune disease. HIES can be triggered by mutation of several immune regulatory proteins (e.g., Tyk2, DOCK8) [60–62]; however, a comparison of symptoms in a subgroup of HIES patients with the roles for STAT3 in the immune system

led 2 independent groups to identify missense and point mutations in *STAT3* that abrogate *STAT3* transcriptional function and subsequent biological responses [63, 64]. The mutations include disruptions in *STAT3* DNA-binding, SH2 and transactivation domains (Figure 2; not shown) [63, 64]. Characteristic features of HIES associated with *STAT3* mutation (*STAT3* AD-HIES) comprise recurring bacterial infections of skin and lungs, enhanced oral fungal infection, elevated innate immune pro-inflammatory responses, bone and connective tissue abnormalities and increased circulating immunoglobulin E (IgE) [65–68]. Key evidence implicating *STAT3* in AD-HIES included upregulation of mRNAs encoding host defense, IFN-inducible and signal transduction components, collectively suggesting altered cytokine responses. In addition, a complex phenotype marked by elevated pro-inflammatory cytokine production upon LPS stimulation, yet defective IL-6-responsiveness, mimics aspects of conditional *Stat3*-deficient mouse models [63, 69, 70].

An important advance in understanding the select organ manifestation of *STAT3* AD-HIES (e.g., impaired skin and lung immunity) was made upon observations that human keratinocytes and bronchial epithelial cells require the combined activity of cytokines from *STAT3*-dependent, IL-17-producing CD4⁺ T lymphocytes (Th17 cells) and pro-inflammatory cytokines to generate antibacterial mediators and neutrophil chemoattractants [71]. By contrast, other cell types activate antimicrobial responses in response to pro-inflammatory cytokines alone. Thus, deficiency in the *STAT3*-dependent Th17 lineage plays a significant role in the tissue-restricted phenotype of *STAT3* AD-HIES.

Evidence of immune system dysfunction in *STAT3* AD-HIES might suggest hematopoietic stem cell transplantation as an effective therapy to treat the disease. Recently, bacterial artificial chromosome (BAC) engineering was used to generate a transgenic mouse model of *STAT3* AD-HIES [72]. These animals overexpress a DNA-binding-defective *STAT3* isoform (*Stat3* V463), which recapitulates several features of the human disease [72]. By testing bone marrow transplantation, Steward-Tharp et al. demonstrated, however, that effective host defense responses require functional *STAT3* in both hematopoietic and non-hematopoietic compartments. In other words, appropriate inflammatory and immune responses were only partially restored by transplantation of *Stat3*-sufficient hematopoietic cells into mice carrying the mutant allele [72]. These data underscore the powerful utility of the *STAT3* AD-HIES mouse as a model to examine molecular and cellular events, as well as communication between immune and non-immune components, that regulate immune competency [72].

STAT3 hyperactivation, autoimmunity and immunodeficiency

Early observations implicating a role for *STAT3* in human autoimmunity centered on association of *STAT3* gene variations (e.g., single nucleotide polymorphisms, SNPs) with increased predisposition to psoriasis and multiple sclerosis; *STAT3* SNPs are also linked with inflammatory bowel disease [73–76]. Recently, de novo activating point mutations in *STAT3* (i.e., GOF) were identified in individuals with juvenile-onset autoimmunity and lymphoproliferation. Disease manifestations include type I diabetes in infancy, lymphadenopathy, autoimmune cytopenias, primary hypothyroidism, solid organ autoimmunity and short stature [77–80]. The *STAT3* point mutations in these individuals

affect conserved residues in the DNA binding, SH2 or transactivation domains, which are different from those mutated in *STAT3* AD-HIES, as well as a residue located in the *STAT3* N-terminal coiled:coil domain (Figure 2; not shown) [77–79]. *STAT3* GOF mutations linked with autoimmunity induce elevated basal and cytokine-responsive *STAT3* transcriptional activity, yet constitutive *STAT3* tyrosine phosphorylation is not detected [77–79]. Biochemical and molecular modeling experiments suggest autoimmunity-associated GOF mutations enhance *STAT3* DNA binding activity, which presumably elevates *STAT3*-mediated transcriptional responses [77]. Furthermore, the activity of other *STATs* is altered upon hyperactivation of *STAT3*, as both *STAT1* and *STAT5* signaling are suppressed in cells expressing autoimmunity-associated *STAT3* mutants. Effects on other *STATs* may explain certain disease features such as short stature or aberrant immune competency, processes that are regulated by growth hormone (GF)-*STAT5* or IFN-*STAT1* signaling, respectively [77–79].

Individuals carrying *STAT3* GOF mutations show reduced regulatory T lymphocytes (Tregs), consistent with roles for *STAT3* in restraining FoxP3 expression and Treg development [81–83], as well as the importance of Tregs in suppressing autoimmunity [84–88]. Surprisingly, however, other immune subsets that were previously reported to be *STAT3*-dependent were also reduced in some individuals with *STAT3* GOF mutations. For example, fewer plasmacytoid dendritic cells (pDCs), natural killer (NK) cells and Th17 cells were detected in certain cases, relative to amounts in healthy controls [78]. Moreover, reduced amounts of class-switched memory B lymphocytes and lower circulating IgG amounts were found, implying coexisting immune defects. One individual carrying an activating *STAT3* mutation developed disseminated mycobacterial disease, which often associates with impaired DC/antigen-presenting populations, and may reflect a more global DC deficiency [78]. A second individual developed T-cell large granular lymphocytic leukemia, indicating the malignant potential of de novo *STAT3* GOF mutations [78]. Overall, a complex phenotype of autoimmunity and specific immune deficiencies presents with *STAT3* GOF mutation [80]. While this disease has yet to be modeled in the murine system for pre-clinical studies, there is encouraging evidence that IL-6 inhibition or bone marrow transplantation may provide treatment options [79].

Conditional deletion of *Stat3* in mice

Stat3 gene deletion in mice is used as a principal approach to evaluate systemic, cellular and molecular roles of *STAT3*. Since germline *Stat3* deletion leads to embryonic lethality [89], conditional *Stat3* ablation using the cre-LoxP system is necessary to study developmental and mature organ functions. Four separate *Stat3* floxed alleles (*Stat3^f*) and corresponding *Stat3^{flox/flox}* (*Stat3^{f/f}*) mouse strains have been generated and analyzed [70, 90–93]. Each *Stat3^f* allele targets distinct regions of *Stat3* for removal, yet in all cases these were designed to eliminate *STAT3* domains necessary for canonical, tyrosine-phosphorylation-mediated transcriptional activity [70, 90, 92, 93]. Here, we provide brief descriptions of the distinct *Stat3^f* alleles, which have been used in numerous reports on *STAT3* immune functions.

Takeda, Akira and colleagues generated a *Stat3^f* allele in which portions of exons 21 and 22 are flanked by loxP sites [70]. Upon cre-mediated deletion, this *Stat3^f* allele encodes an

internally deleted STAT3 protein missing 29 amino acids, including Y705 and S727. As expected, this mutant protein fails to undergo cytokine-responsive tyrosine phosphorylation and is predicted to be deficient in serine phosphorylation [70]. A very low level of a truncated STAT3 protein can be observed in cre-expressing hematopoietic and immune cells containing 1 or 2 copies of this *Stat3^f* allele. Based on the protein coding regions targeted and cross-reactivity with C-terminal-specific antibodies, the truncated STAT3 protein appears to result from an in-frame internal deletion due to the location of the loxP sites [70, 94]. While it is formally possible that a low amount of the internal deletion STAT3 mutant has certain unknown biological function, this *Stat3^f* strain recapitulates data obtained with distinct *Stat3^f* alleles [69, 92, 93], indicating that it permits full ablation of canonically activated STAT3.

Raz, Lee, Levy and colleagues developed a *Stat3^f* allele with loxP sites bracketing exons 16–21 [90, 91]. Cre-mediated excision of these floxed exons removes sequences encoding the STAT3 SH2 domain and Y705, and renders complete absence of tyrosine-phosphorylated as well as full-length STAT3 protein [90, 91]. Although the STAT3 S727- or TAD-encoding sequences were not removed through this approach, the targeting construct does not generate an in-frame internal STAT3 deletion and thus effectively eliminates all STAT3 sequences C-terminal to exon 21 [90,91].

Welte et al generated a *Stat3^f* allele in which exons 18–20 are flanked by loxP sequences [92]. Removal of exons 18–20 upon cre expression leads to deletion of the STAT3 SH2 domain, which is expected to eliminate transcriptional activity dependent on STAT3 Y705 phosphorylation and STAT3 homodimerization. STAT3 protein was not observed in immunoblotting assays with antibodies that recognize the C-terminus of STAT3 [92], implying a lack of in-frame sequences beyond exon 20 (e.g., encoding Y705, S727 and TAD). These data indicate efficient depletion of canonically activated STAT3 protein [92].

Alzoni, Poli and colleagues developed a *Stat3^f* allele with loxP sites flanking exons 12 to 14, which encode the DNA-binding domain of STAT3 [93]. This *Stat3^f* allele generates a truncated and frameshifted mRNA, predicted to encode a STAT3 protein deficient in DNA-binding. Thus, this approach should ablate both canonically activated (tyrosine-phosphorylated) as well as uSTAT3 transcriptional functions. STAT3 protein levels produced from this *Stat3^f* allele upon cre expression were greatly reduced and *Stat3*-deficient animals recapitulated phenotypes observed with other mutant *Stat3* strains, indicating effective deletion of STAT3 [93].

Since homozygosity (*Stat3^{f/f}*) of each of the 4 *Stat3^f* alleles renders severe reduction or non-detectable amounts of tyrosine-phosphorylated, full-length STAT3 in cre expressing cells [70, 90–93, 95], we refer to these strains collectively as *Stat3^{f/f}* or *Stat3*-deficient. Nonetheless, it is important to understand the *Stat3^f* allele utilized in various literature reports due to new information on roles for uSTAT3, mitochondrial STAT3 and potential for residual canonical or non-canonical function in the absence of complete protein depletion.

STAT3 in innate immunity

Emergency and steady state granulopoiesis

STAT3 regulates critical steps during emergency granulopoiesis, a key innate response elicited upon bacterial or fungal invasion that provides a rapid increase in the supply of circulating neutrophils to help contain infection. Emergency granulopoiesis involves both the swift release of mature neutrophils from the bone marrow reserve as well as the induction of granulopoiesis de novo to sustain elevated neutrophil output [96]. Elegant studies indicate that initial pathogen encounter with endothelial cells (ECs) stimulates ECs to synthesize G-CSF, the major neutrophil growth factor, which then induces granulocytic progenitor proliferation and neutrophil mobilization responses in bone marrow [97–99]. Importantly, emergency granulopoiesis can be mimicked by delivery of supraphysiologic amounts of recombinant G-CSF, a situation also encountered clinically during G-CSF treatment of immunocompromised individuals [100].

STAT3 is the primary STAT protein activated upon G-CSF engagement with G-CSFR [101]. Initial evidence for the involvement of STAT3 in emergency granulopoiesis was obtained by study of the *Tg(Tek-cre)12Flv Stat3^{fl/f} mice* (*Tek-cre Stat3^{fl/f}*, also known as Tie2 cre *Stat3^{fl/f}*), in which cre expression is driven by the *Tek/Tie2* promoter. These animals have been used as a model to understand hematopoietic STAT3 function [94,102–106]. Upon systemic administration of G-CSF or delivery of the chemokine MIP-2, an agonist for the neutrophil chemoattractant receptor CXCR2, *Tek-cre Stat3^{fl/f}* mice fail to mobilize mature neutrophils from the bone marrow effectively, relative to *Stat3*-sufficient controls. Furthermore, FACS-purified *Stat3*-deficient neutrophils from *Tek-cre Stat3^{fl/f}* mice are impaired in their ability to undergo chemotaxis toward CXCR2 ligands (i.e., MIP-2, KC) ex vivo [94,104]. These data indicate STAT3 regulates neutrophil release to the circulation and CXCR2-mediated chemotaxis needed to access infected tissues, which are key steps in emergency granulopoiesis. *Tek-cre Stat3^{fl/f}* mice also fail to upregulate immature granulocyte amounts in bone marrow or peripheral blood. This defective response reflects a vital role for STAT3 in G-CSF-driven proliferation of granulocytic progenitor cells, which undergo rapid expansion during emergency granulopoiesis [94,103]. It is critical to recognize, however, that *Tek-cre Stat3^{fl/f}* animals also have endothelial *Stat3*-deficiency due to the Tie2 expression pattern. While this has potential to contribute to observed phenotypes, data from ex vivo proliferation and chemotaxis assays, as well as the identification of STAT3 target genes mediating emergency granulopoiesis, imply key hematopoietic cell-intrinsic roles for STAT3 [94,103].

During the proliferative response to G-CSF in emergency granulopoiesis, STAT3 stimulates expression of C/EBP β and c-Myc in bone marrow granulocytic progenitors; these regulators have critical functions in driving enhanced G1/S phase progression and induction of the emergency response (Figure 3) [103,107,108]. Molecular studies indicate STAT3 mediates transcriptional activation of *Cebpb* and *c-myc* directly [103]. Moreover, STAT3-dependent upregulation of C/EBP β also promotes C/EBP β association at the *c-myc* promoter. The concurrent association of STAT3 and C/EBP β at the *c-myc* promoter displaces the negative regulator C/EBP α , resulting in enhancement of *c-myc* transcription

[103,107]. STAT3 also stimulates expression of CXCR2/*Il8rb* and MIP-2/*Cxcl2* by direct promoter binding upon G-CSF stimulation to augment neutrophil migratory capability [104,109].

The aforementioned responses are involved in driving emergency granulopoiesis, however, STAT3 is correspondingly required to restrain neutrophil production and limit inflammatory responses [91,110]. A key negative regulatory mechanism is directed by STAT3-dependent induction of SOCS3, an important signaling intermediate that suppresses the activity of the G-CSFR receptor (G-CSFR) as well as a subset of other cytokine receptors [91,111–113]. STAT3 stimulates *Socs3* transcription rapidly upon cytokine engagement, from nearly undetectable amounts in basal conditions to a greater than 10-fold increase in mRNA and protein expression [114]. Accumulated SOCS3 protein in the cytoplasm interacts with receptor/Jak complexes through two distinct interfaces simultaneously, resulting in inhibition of receptor-mediated signal transduction. The central SH2 domain of SOCS3 binds to the activated (tyrosine-phosphorylated) receptor intracellular region via a classic SH2-phosphotyrosine interaction. In parallel, the kinase-inhibitory region (KIR) of SOCS3 located near the N-terminus associates with the receptor-associated Jak protein, binding within the Jak-substrate interaction groove, which inhibits the Jak kinase from association with other substrates [115,116]. These SOCS3-mediated mechanisms have been established by study of IL-6 receptor signaling, and are thought to extend to G-CSFR as well as specific other cytokine receptors effectively blocked by SOCS3 [112,113,115–117].

Collectively, therefore, information from numerous murine models indicates STAT3 orchestrates key proliferative and neutrophil migratory functions required for emergency granulopoiesis, and also limits the duration of this response to prevent destructive inflammation. Furthermore, *Tek-cre Stat3^{fl/fl}* animals have defective clearance of *Listeria monocytogenes*, while *Stat3*-deficient neutrophils and macrophages show impaired bactericidal activity, indicating critical STAT3 antibacterial functions [104,118]. These activities of STAT3 maybe conserved, since *STAT3* AD-HIES individuals are susceptible to certain bacteria and isolated neutrophils show migration impairments [94,104,119–122]. Further investigation is needed to examine roles for STAT3 in mediating human neutrophil responses.

In steady state, G-CSF and G-CSFR also serve as the major ligand-receptor pair controlling neutrophil development [98,101,123–126]. Thus it was expected STAT3 would be required to mediate neutrophil production in homeostasis. Surprisingly, however, animals with *Stat3* deletion in hematopoietic cells (e.g., *Tek-cre Stat3^{fl/fl}* mice or global *Mx-cre*-mediated *Stat3* deletion) show elevated amounts of circulating neutrophil numbers [91, 92, 94]. The key mechanism thought to underlie this excessive neutrophil accumulation centers on STAT3-dependent control of SOCS3, since *Stat3*-deficiency as well *Socs3*-deficiency in the hematopoietic system leads to neutrophilia [91, 111]. STAT3-mediated regulation of neutrophil chemotactic factors may also prevent effective neutrophil margination in tissues [94,104,109,127], contributing to neutrophil accumulation in the circulation, yet this hypothesis must be further examined.

STAT3-responsive pathways in developing granulocytes are affected by other cytokine cues, with significant impact upon granulopoiesis. Type IIFN (IFN- γ), which is produced by activated CD4⁺ and CD8⁺ T lymphocytes as well as NK and NKT cells, preferentially stimulates monocyte generation from the granulocyte-monocyte progenitor (GMP) population, at the expense of granulocytic cell production [128,129]. The action of IFN- γ on monocytes and macrophages is critical for enhancing immunity toward intracellular bacteria [130], thus IFN- γ -driven monocyte generation can be viewed as a feedforward mechanism to promote a dedicated immune response for this specific pathogen class. IFN- γ potently inhibits STAT3 activation in myeloid progenitor cells while simultaneously inducing genes required for monocyte differentiation (e.g., PU.1, IRF8). IFN- γ -dependent STAT3 inhibition is thought to be regulated via IFN- γ -responsive SOCS3 induction and subsequent inhibition of G-CSFR-STAT3-mediated proliferation within the GMP and common myeloid progenitor (CMP) populations [129]. Thus, cytokine-driven stimulation or inhibition of STAT3 activity in myeloid progenitors may be key to tailoring hematopoietic output, to meet unique demands upon the immune system during different infection or emergency conditions (Figure 3) [103,129,131].

Dendritic cell (DC) development and function

DCs comprise several distinct populations that are identified by unique cell surface marker expression, sites of anatomical residence and functional responses to damage- and pathogen-associated molecular patterns (DAMPs and PAMPs, respectively) elicited in tissue injury and infection [132,133]. The classic division in DC lineages is drawn between the plasmacytoid DCs (pDCs), professional type IIFN producing cells, and conventional DCs, which reside in lymphoid and non-lymphoid tissues and have potent phagocytic and antigen presenting activities [132,133].

Initial work with *Tek-cre Stat3^{fl/fl}* mice indicated a critical role for STAT3 in development of pDC and cDC lineages [102]. This function was particularly evident in DC generation driven by administration of exogenous Fms-related tyrosine kinase 3 (Flt3) ligand (Flt3L), the major DC growth factor in vivo [102,134]. These data agree with selective STAT3 activation (versus other STATs) by Flt3L, as well as the critical role for STAT3 in stimulating Flt3L-responsive DC progenitor proliferation [106,135]. Recently, prostaglandin E2 (PGE2) has been implicated in optimal Flt3L-mediated DC generation via a STAT3-dependent pathway [136], indicating non-cytokine signals can converge on DC progenitors to drive STAT3 activation and DC production.

Studies employing overexpression of STAT3 or Flt3 (the receptor for Flt3L) within Flt3-negative hematopoietic progenitors, which normally do not have the ability to produce DCs, implicated key instructive functions for these factors in DC development [137,138]. For example, constitutive Flt3 expression promotes induction of genes involved in DC development (*Stat3*, *Spi1/PU.1*) and enhances pDC and cDC generation [138]. Similarly, STAT3 overexpression stimulates Flt3 expression and DC development [138]. Additional work implicated STAT3 in driving *Spi1/PU.1* expression [127,139]. Furthermore, PU.1 is critical for Flt3 expression and DC development in vivo [140]. These data collectively

suggest the existence of a positive, feedforward molecular pathway mediated by Flt3L-Flt3-STAT3 signaling and PU.1 that promotes DC development [138,140,141].

More recent work with *Tek-cre Stat3^{fl/f}* or *Tg(Itgax-cre)1-Reiz Stat3^{fl/f}* (*Itgax-cre*, also known as CD11c cre) animals confirms the crucial role for STAT3 in pDC generation, yet indicates steady state production of lymphoid organ CD8 α ⁺ cDCs and nonlymphoid resident CD103⁺ DCs is STAT3-independent [106,142]. Since *Stat3* deletion is restricted to CD11c⁺ populations in *Itgax-cre Stat3^{fl/f}* mice, it is possible cDCs require STAT3 only before reaching a CD11c-expressing, DC-committed precursor stage. By contrast, a likely explanation for the differences obtained in separate studies of *Tek-cre Stat3^{fl/f}* mice may center on the severe inflammatory disease that develops in these animals, which causes early lethality (e.g., 6–8 weeks) in specific pathogen-free housing conditions [92, 94]. Systemic inflammation may have deleterious consequences on hematopoietic stem and progenitor populations, indirectly affecting DC output. Further studies to explore cell autonomous roles for STAT3 in homeostatic DC generation are needed to examine this issue more carefully.

In addition to STAT3, pDC development is critically dependent upon the basic helix-loop-helix (bHLH) transcriptional regulator E2-2 [143]. Conversely, pDC generation and pDC-mediated type IIFN production are suppressed by inhibitor of differentiation 2 (*Id2*), a member of the *Id* protein family that blocks the DNA binding activity of bHLH-containing transcription factors including E2-2 [144,145]. These findings raised the question of whether STAT3 and/or other STATs control DC lineage-regulatory factors. Experiments using *Tek-cre Stat3^{fl/f}* mice demonstrated a critical role for STAT3 in mediating Flt3L-responsive *Tcf4/E2-2* expression in common DC progenitors (CDPs). Moreover, molecular assays indicate STAT3 directly stimulates *Tcf4/E2-2* transcription. Taken together, these results suggest the Flt3L-Flt3-STAT3 pathway promotes pDC generation from CDPs via direct induction of *Tcf4* [106,146]. By contrast, GM-CSF and STAT5 upregulate *Id2* in CDPs and inhibit pDC development [106]. These studies reveal cytokine-STAT pathways that influence commitment to specific DC lineages, yet they are likely to reflect only a small fraction of STAT3- (or other STAT-) driven molecular responses. Global STAT3 transcriptional profiling and STAT3 chromatin association studies from defined hematopoietic progenitors are needed to fully appreciate STAT3 mechanisms in DC development.

Numerous reports indicate STAT3 suppresses DC maturation and activation, and promotes tolerogenic function [147–151]. This response is attributed to inhibition of MHC class II and co-stimulatory molecule expression; upregulation of myeloid-related protein SA100A9, which suppresses DC function; induction of inhibitory programmed death ligand-1 (PD-L1) on DCs; and STAT3-mediated restraint of TLR-induced pro-inflammatory mediators [152–155] [142]. Repression of DC maturation/function can be achieved via IL-6-STAT3 or IL-10-STAT3-mediated signaling directly, or indirectly through inhibitory molecules that induce IL-6 [152,156,157]. Blockade of STAT3 reverses many of these immunosuppressive responses, which may have particularly important consequences in rewiring tolerogenic tumor microenvironments for improved tumor clearance [151,158–160].

STAT3 anti-inflammatory signaling in phagocytes

STAT3 has a key role in suppressing signal transduction mediated by Toll-like receptors (TLRs), most notably TLR4, TLR2 and TLR9, in mature phagocytic cells [69,142]. For example, *Stat3*-deficient macrophages, neutrophils and DCs produce elevated amounts of pro-inflammatory cytokines upon TLR4 activation, including TNF- α , IL-6, IL-12, and IFN- γ . While these populations also produce excessive amounts of IL-10, they lose responsiveness to this anti-inflammatory cytokine, which inhibits TLR4-dependent pro-inflammatory cytokine production [69,142]. Mice with hematopoietic-wide *Stat3*-deficiency (*Tek-cre Stat3^{fl/fl}*), DC-restricted *Stat3*-deficiency (*Itgax-cre Stat3^{fl/fl}*), or *Stat3*-deficiency in macrophages and neutrophils (*Lyz2^{tm1(cre)Ifi} Stat3^{fl/fl}*, also known as *Lyz2-cre Stat3^{fl/fl}* or *LysM cre Stat3^{fl/fl}*) have increased amounts of circulating pro-inflammatory cytokines and develop mild to severe enterocolitis in early adulthood [69, 92, 93,142]. Intestinal inflammation in these *Stat3*-deficient strains is accompanied by enhanced activity of IL-12-dependent T helper 1 (Th1) cells, as evidenced by elevated Th1-mediated IFN- γ production [69, 142].

Significantly, enterocolitis was improved in *Lyz2 Stat3^{fl/fl}* mice upon concomitant deletion of *Tlr4* or *Il12b* (encoding IL-12p40), but not *Stat1* or *Tnfa* [161]. Treatment with IL-12p40 neutralizing antibodies or simultaneous *Rag1*-deficiency also suppresses intestinal inflammation [93,162]. These results indicate critical roles for IL-12 as well as innate and adaptive immune populations in mediating inflammatory disease. Furthermore, *Il10*-deficient animals develop a chronic intestinal inflammatory disease similar to that observed in the hematopoietic-wide, DC- or myeloid-restricted *Stat3*-deficient animals [163]. Taken together, these data underscore the key protective nature of IL-10 and STAT3 signaling in the intestine, and imply persistent stimulation from the intestinal microbiota induces excessive cytokine production in intestinal phagocytes via unchecked TLR4 signaling, resulting in an IL-12-dependent Th1-mediated inflammatory disease [93,161,162]. This model is consistent with studies demonstrating a critical role for phagocytic-specific TLR-MyD88 signaling in driving intestinal inflammation when unopposed by IL-10 [164]. Interestingly, aged *Tlr4-null Lyz2 Stat3^{fl/fl}* mice show evidence of intestinal inflammatory disease, suggesting excessive signaling via other TLRs (e.g., TLR9) contributes to pathology [161].

The critical role for STAT3 in mediating the anti-inflammatory effects of IL-10 was firmly established by several groups [165–168], yet the cell intrinsic mechanism whereby STAT3 restrains pro-inflammatory gene expression has been elusive for nearly a decade. Early studies indicated regulation at the transcriptional level [169]. Pro-inflammatory genes are targets of NF- κ B and MAPK signaling cascades and, in some cases, also regulated by IRF3/7; however, there is little evidence for direct STAT3 interference at the wide array of pro-inflammatory gene promoters. By contrast, STAT3 was found to induce expression of transcriptional repressors and co-repressors that inhibit NF- κ B gene reporters [170], suggesting an indirect mechanism by which STAT3 restrains pro-inflammatory gene transcription. Other potential anti-inflammatory effectors have been identified [171–177]. Nonetheless, the impact of these pathways on the broad STAT3 anti-inflammatory effect remains to be tested with genetically-modified mouse models and in vivo studies.

Key insight into the cell intrinsic STAT3 anti-inflammatory mechanism was obtained recently by studies in *Tek-cre Stat3^{fl/fl}* mice [178]. In addition to developing severe intestinal inflammation, these animals have decreased calcified bone and elevated amounts of osteoclasts. Furthermore, *Stat3*-deficient bone marrow-derived macrophages exhibit an enhanced propensity to develop osteoclasts upon Receptor Activator of NF- κ B (RANK) stimulation ex vivo. These data collectively suggest *Stat3*-deficient macrophages/osteoclast precursors have increased RANK responsiveness. Since RANK and TLR4 utilize similar signal transduction cascades, culminating in NF- κ B and MAPK activation, this prompted investigation into core signaling factors in *Stat3*-deficient macrophages.

STAT3 was found to inhibit expression of a key E2 ubiquitin-conjugating enzyme, Ubc13, required for RANK and TLR4 signaling. STAT3 controls Ubc13 expression by direct transcriptional repression of *Ube2n*, the gene encoding Ubc13 [178]. Significantly, the accumulation of Ubc13 in *Stat3*-deficient macrophages has a central, non-redundant role in mediating enhanced RANK- and TLR4 signaling in the absence of STAT3. Therefore, taken together, these data indicate STAT3 exerts a broad suppressive function upon NF- κ B and MAPK activity in macrophages by restraining Ubc13 abundance through *Ube2n* transcriptional inhibition (Figure 4) [178]. These results are consistent with the global repression of macrophage proinflammatory gene expression mediated by STAT3 [169]. Furthermore, they suggest excessive STAT3 activity may aberrantly induce anti-inflammatory responses. In agreement, IL-6 was shown to stimulate an atypical anti-inflammatory response in *Socs3*-deficient cells, which show unrestrained and prolonged STAT3 activation [179,180]. While the discovery of the STAT3-Ubc13 pathway reveals a key cell intrinsic anti-inflammatory mechanism in macrophages, additional work is needed to examine the role of this pathway in cytokine-mediated anti-inflammatory signaling in vivo.

Significantly, STAT3 anti-inflammatory function appears to be conserved between mice and humans. Elevated basal and TLR4-responsive expression of pro-inflammatory cytokines was found in peripheral blood neutrophils and mononuclear cells from individuals with *STAT3* AD-HIES [63], suggesting human STAT3 restrains pro-inflammatory gene expression. Moreover, the STAT3 binding site within the *Ube2n/UBE2N* promoter is highly conserved, which suggests potential for a STAT3-Ubc13 anti-inflammatory mechanism in humans [178]. Disordered inflammation is a prominent characteristic of *STAT3* AD-HIES, consistent with elevated pro-inflammatory signaling and severely impaired IL-10 responses [181,182]. In addition, individuals with *STAT3* AD-HIES exhibit skeletal abnormalities and propensity for bone fractures with mild trauma, suggesting STAT3 may regulate bone homeostasis in humans via Ubc13 restraint, similar to mice [67,178,183]. Nonetheless, whether the STAT3-Ubc13 pathway is key to human anti-inflammatory responses requires further examination.

STAT3 anti-inflammatory signaling in non-immune populations

A handful of evidence implicates anti-inflammatory roles for STAT3 in non-immune populations, suggesting this function of STAT3 may operate in numerous tissues in homeostasis and/or disease. For example, STAT3 signaling within endothelial cells, tumor cells (e.g., melanoma) or fibroblasts suppresses production of proinflammatory factors

[148,184,185]. Whether this inhibition occurs via Ubc13 restraint remains to be established, although Ubc13 is broadly expressed and further increased in *Stat3*-deficient fibroblasts, suggesting the potential for a parallel STAT3-Ubc13 anti-inflammatory mechanism in non-immune cells [178]. Moreover, STAT3 mediates an additional protective role in the intestine by regulating epithelial cell homeostasis, mucosal wound healing and mucus production during experimental colitis. This mechanism involves secretion of IL-22 by innate immune cells and subsequent IL-22-mediated STAT3 signaling within intestinal epithelial cells [186–188], indicating beneficial crosstalk between immune and non-immune cells involving STAT3.

STAT3 regulation of adaptive immunity

B lymphocytes

B cells serve multiple important roles in immunity, including immunoglobulin production, antigen presentation and T lymphocyte helper functions. Deletion of *Stat3* affects numerous B cell activities. *Stat3* removal using the interferon-inducible *Mx-cre* transgene led to fewer B cells in bone marrow and peripheral tissues versus control animals. These studies showed STAT3 is required for developmental transition of the pre-pro-B cell progenitor to subsequent precursor populations, as well as precursor survival (Figure 5) [189]. This phenotype may reflect the dependence of early B cell development on Flt3L, which induces STAT3 signaling in Flt3⁺ hematopoietic progenitor cells including common lymphocyte progenitors (CLPs) [134,135]. Moreover, IL-7-responsive progenitors were reduced in the absence of STAT3 without effects upon IL-7 receptor (IL-7R) signaling [189], consistent with an upstream defect in progenitor development (e.g., via a Flt3L-STAT3-dependent stage) and the primary use of STAT5 by the IL-7R.

By contrast, STAT3 deletion from later stage B lineage-committed CD19⁺ precursors using *Cd19-cre Stat3^{fl/fl}* mice (i.e., deleted at pro-B, pre-B and subsequent developmental stages) demonstrated a critical role for STAT3 in the differentiation of immunoglobulin G (IgG) secreting plasma cells [190]. This process requires T lymphocytes producing IL-21, which elicits STAT3 (and STAT1) signaling in B cells (Figure 5). IL-21-induced STAT3 stimulates expression of the B cell maturation factor Blimp-1 and thereby drives IgG-producing plasma cell generation [191–194]. Molecular studies indicate STAT3 binds the *Prdm1/Blimp1* locus in B cells, displacing the negative regulator BCL6; moreover, this transcriptional mechanism is enhanced upon CD40L stimulation [193]. STAT3 may also have a role in IL-35-mediated induction of IL-10- and IL-35-secreting B regulatory cells (Bregs), due to its responsiveness to IL-35 receptor signaling (i.e., via IL-12Rβ2 and IL-27Rα subunits) [195]. Bregs are implicated in suppression of host defense, autoimmunity and anti-tumor responses [195]. Thus, data to date collectively imply functions for STAT3 in both activation and repression of B cell-mediated adaptive immunity, mirroring stimulatory and inhibitory roles for STAT3 in innate immunity.

Significantly, studies of *STAT3* AD-HIES show STAT3 regulates human B effector and memory populations, including IL-21-dependent plasma cells [196, 197]. The humoral response in *STAT3* AD-HIES maybe further compromised by defective T follicular helper (Tfh) cell generation, as Tfh production also requires STAT3 (discussed further below)

[198,199]. It is critical now to understand how STAT3 control of IL-35 responses and Breg functions factor into the *STAT3* AD-HIES disease phenotype. In addition, the unexpected finding that STAT3 GOF mutations lead to fewer class-switched memory B lymphocytes and reduced circulating IgG in individuals with associated autoimmunity implies as yet unrevealed immunological mechanisms, which must be explored further.

CD4⁺ T lymphocytes

In response to antigen stimulation via the T cell receptor (TCR) and specific cytokine cues, naïve CD4⁺ T cells develop into distinct effector subsets with unique immune functions including CD8⁺ T cell activation, stimulation of innate immune cells and induction of B cell responses [200]. Early evidence from mice with T cell-specific *Stat3* deletion (*Lck-cre Stat3^{fl/fl}*) indicated a crucial function for STAT3 in IL-6-mediated T cell survival, independent of Bcl2 regulation [70, 201]. Subsequently, STAT3 was found to be essential for induction of IL-17-producing (Th17) cells from naïve CD4⁺ precursors (Figure 5). Th17 generation is driven by IL-6- and TGF- β , as well as an autocrine IL-21 signaling cascade, upon TCR activation [202–205]. STAT3 is specifically required for responses to IL-6 and IL-21. At the molecular level, STAT3 stimulates expression of the Th17 lineage-specifying factors retinoic acid receptor-related orphan receptors gamma and alpha (ROR γ and ROR α), which are required for Th17 development. STAT3 also upregulates IL-23 receptor (IL-23R) and IL-17 expression. IL-23R signaling enhances Th17 development in the presence of IL-23, while secreted IL-17 executes canonical effector functions of the Th17 subset [203, 205–209]. Th17 cells are critical for host defense to extracellular and intracellular bacteria, as well as fungi, yet are also involved in numerous inflammatory and autoimmune diseases. These functions are mediated primarily by IL-17, which stimulates production of immune effectors such as anti-microbial peptides, chemokines and granulopoietic cytokines [208, 209]. Thus, STAT3 mediates neutrophil-driven inflammatory responses by direct effects on granulopoiesis and via regulation of the Th17 lineage.

STAT3 also controls development of Tfh cells. This population is characterized by expression of the CXCR5 chemokine receptor, localization to the B cell follicle within germinal centers of secondary lymphoid organs and IL-21 secretion. IL-21 production from Tfh cells has a key role in mediating B cell “help” in germinal centers by stimulating proliferation and antibody affinity maturation, as discussed above. Furthermore, Tfh cell generation is dependent upon IL-6- or IL-21-responsive STAT3 signaling (Figure 5), which upregulates expression of BCL6, a lineage-specifying transcriptional regulator [210, 211]. STAT3 also mediates IL-27 signaling within developing Tfh cells to stimulate IL-21 production, T cell survival and expression of Tfh phenotypic markers [212]. Therefore, STAT3 has critical roles within both T and B cell compartments that culminate in production of plasma cells and IgG secretion.

By contrast to these lineage-inducing events, STAT3 potentially inhibits generation of CD4⁺ T regulatory cells (Treg) from naïve CD4⁺ precursors and suppresses expression of the Treg-specifying transcription factor Foxp3 in mature Tregs (Figure 5) [83, 213]. In the setting of graft-versus-host disease (GVHD), *Stat3*-deficiency promotes inducible Treg generation, restrains GVHD and improves survival [83]. These data suggest STAT3 blockade in CD4⁺ T

cells may be useful in treating GVHD. Moreover, STAT3 and Foxp3 appear to co-regulate specific genes in differentiated Tregs, including *Il10*, as indicated by expression studies in *Foxp3-cre Stat3^{fl/fl}* versus wild type Tregs [214]. In this context, STAT3 has a key antiinflammatory role by maintaining the ability of Foxp3⁺ Tregs to inhibit inflammatory Th17 cells [214–216].

Interestingly, STAT3 and STAT5 have mutually antagonistic activity in IL-2-induced Treg and IL-6-induced Th17 generation, respectively [202]. These results are explained at least in part by unique patterns of STAT binding and activity at loci encoding the Treg lineage-specifying factor Foxp3 (*Foxp3*) and the Th17-specific cytokine IL-17 (*Il17*). For example, *Foxp3* expression is stimulated directly by IL-2-activated STAT5, while inhibited by IL-6-activated STAT3 [81, 213, 217]. On the contrary, *Il17* expression is driven by IL-6-stimulated STAT3, which binds multiple sites in the *Il17* locus. Upon IL-2 stimulation, however, STAT5 associates with common/overlapping sites, resulting in decreased STAT3 association. STAT5 binding in the *Il17* locus is accompanied by a reduction in chromatin marks that reflect active transcription and lower *Il17* mRNA expression [217]. These data imply divergent roles for STAT3 and STAT5 in transcriptional activation or repression of *Il17*, respectively. Collectively, the opposing functions for STAT3 and STAT5 at the transcriptional and epigenetic levels induce distinct developmental outcomes from naïve CD4⁺ T cells, contributing essentially to the diversity of CD4⁺ T cell subsets [200].

Importantly, STAT3 has key conserved functions in regulating human Th17 and Tfh cells; loss of these activities in *STAT3* AD-HIES contributes significantly to the disease phenotype [199, 218]. Yet, unexpectedly, STAT3 LOF also reduces inducible Tregs in humans. This phenotype may be due to impaired IL-10 signaling in DCs [218]. Moreover, as discussed above, early studies of humans with STAT3 GOF-mediated diseases have revealed surprising deficits in cells that exhibit STAT3-dependency in mice [78, 80]. Since many immune responses depend on interaction of multiple cell types, often mediated by cytokines, it is imperative to dissect primary and secondary responses in STAT3 LOF and GOF-associated disease.

CD8⁺ T lymphocytes

Cytotoxic CD8⁺ T cells (CTLs) are critical for clearing cells infected with intracellular pathogens, typically viruses, as well as cells expressing aberrant host factors such as oncoproteins. The direct cytotoxic activity of CD8⁺ T cells in tumors is frequently associated with better prognosis and improved tumor clearance, generating significant interest in understanding how to further promote this response via conventional cancer therapy and/or immunotherapy. Naïve CD8⁺ T cells differentiate into potent effector cells, which in turn generate long-lived memory cells. While both CD8⁺ T cell effector and memory responses are regulated transcriptionally, STAT3 has critical roles in generating stable, long-lived memory cells (Figure 5) [219, 220]. STAT3 controls expression of the CD8⁺ T cell transcriptional regulators Eomes, BCL-6 and Blimp-1, as well as the cytokine signaling inhibitor SOCS3, as indicated by their reduction in *Stat3*-deficient memory CD8⁺ T cells. Moreover, the absence of SOCS3 is associated with CD8⁺ T cell hyperresponsiveness to IL-12. These data suggest STAT3-SOCS3 signaling may protect

CD8⁺ T memory precursors from cytokine cues that regulate CD8⁺ T effector differentiation [219]. Upstream, the cytokines IL-10 and IL-21 play critical roles in activating STAT3 to drive formation of stable memory CD8⁺ T cells [219]. Recent data indicate the memory T response is enhanced by IL-10 secretion during the resolution phase of infection [221]. Importantly, individuals with *STAT3* AD-HIES also demonstrate reduced amounts of central memory CD8⁺ T cells, as well as fewer memory CD4⁺ T cells, relative to healthy controls [222]. This likely contributes significantly to the impaired ability of individuals with *STAT3* AD-HIES to manage certain chronic infections [222]. Furthermore, similar to their murine counterparts, human naïve T cells with *STAT3*-deficiency exhibit impaired proliferation and have reduced expression of BCL6 and SOCS3 [222, 223], implying highly conserved pathways in memory T cell generation.

Conclusions and future perspectives

While STAT3 functions are broadly recognized in the immune system, we lack insight into whether and how STAT3 regulates newly discovered or less abundant populations, such as innate immune lymphocytes or other granulocytic subsets. Recently, STAT3 was linked with mast cell degranulation and protection from allergic disease [224], implying additional activities to be discovered. These will be important to uncover in light of the development of STAT3 inhibitors for clinical use.

Moreover, STAT3-mediated responses in innate and adaptive immune subsets appear to have key roles in tumorigenesis, yet critical insight into cell type-specific activities is still needed. Significantly, many tumor types produce STAT3-stimulatory cytokines such as IL-6, G-CSF or vascular endothelial growth factor.

Enhanced STAT3 activation in bone marrow progenitors by the combined actions of IL-6 and G-CSF induce neutrophil generation with elevated pro-tumor responses and concomitant suppression of neutrophil functions associated with anti-tumor activity [225], indicating critical tumor-immune crosstalk. Lastly, it is important to highlight results that indicate persistently activated STAT3 within cancer cells is associated with malignancy. Chronic STAT3 activity is induced via several mechanisms, including tumor-specific STAT3 GOF mutations, hyperactivation due to kinase activities (e.g. KRAS) and local cytokine production. Therefore, modulating STAT3 activity to a level that supports immunity but prevents disease-promoting mechanisms may prove to be essential to the success of STAT3 inhibitors in the clinic.

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Abbreviations

APRF	acute phase response factor
BAC	bacterial artificial chromosome

bHLH	basic helix-loop-helix
Breg	B regulatory cells
CDPs	common DC progenitors
CLPs	common lymphocyte progenitors
CMP	common myeloid progenitor
CTLs	cytotoxic CD8 ⁺ T lymphocytes
DAMPs	damage-associated molecular patterns
eIF2a	eukaryotic translation initiation factor 2a
EIF2AK2/PKR	eIF2a kinase 2/protein kinase R
ES cells	embryonic stem cells
DC	dendritic cell
Flt3	Fms-related tyrosine kinase 3
Flt3L	Flt3 ligand
G-CSF	granulocyte colony-stimulating factor
G-CSFR	G-CSFR receptor
GAS	IFN- γ -activated sequence element
GOF	gain-of-function
GMP	granulocyte-monocyte progenitor
GVHD	graft-versus-host disease
HIES	hyper IgE syndrome
IFN	interferon
IL	interleukin
IL-6	interleukin-6
IL-7	interleukin-7
IL-7R	IL-7 receptor
IL-23	interleukin-23
IL-23R	IL-23 receptor
IgE	immunoglobulin E
IgG	immunoglobulin G

Id2	inhibitor of differentiation 2
KIR	kinase-inhibitory region
LOF	loss-of-function
MAPK	mitogen-activated protein kinase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer cell
PAMPs	pathogen-associated molecular patterns
pDCs	plasmacytoid dendritic cells
PD-L1	programmed death ligand-1
PGE2	prostaglandin E2
PI3K	phosphoinositol 3-kinase
RANK	Receptor Activator of NF- κ B
RORa	retinoic acid receptor-related orphan receptor alpha
RORγ	retinoic acid receptor-related orphan receptor gamma
ROS	reactive oxygen species
SH2	Src homology 2
SNPs	single nucleotide polymorphisms
STAT	Signal Transducers and Activators of Transcription
STAT3 AD-HIES	HIES associated with <i>STAT3</i> mutation
<i>Stat3</i>^{ff}	<i>Stat3</i> ^{flox/flow}
TCR	T cell receptor
Tfh	T follicular helper
Th1	CD4 ⁺ T helper 1 lymphocytes
Th17	IL-17-producing CD4 ⁺ T lymphocytes
TLRs	Toll-like receptors
Tregs	regulatory T lymphocytes
uSTAT3	unphosphorylated STAT3

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Biographies

Emily J. Hillmer received her B.A. in Biology from Carleton College in 2015. She is conducting a research internship with Dr. Watowich in the Department of Immunology at MD Anderson Cancer Center. Her studies are investigating the molecular mechanisms by which STAT3 protects hematopoietic stem cells from damaging pro-inflammatory signals.

Huiyuan Zhang received her M.D. from Beijing University of Chinese Medicine in 2000 and her Ph.D. in Immunology from the Chinese Academy of Medical Sciences & Peking Union Medical College in 2005. Dr. Zhang is an Instructor in the Department of Immunology at MD Anderson Cancer Center. Her research focuses on cytokine and STAT-mediated control of hematopoietic stem cells and myeloid lineages in homeostasis, inflammatory disease and cancer.

Haiyan S. Li received her M.D. from Beijing Medical University in 1998, her M.P.H. degree from Hadassah Medical School at The Hebrew University in 2000, and her Ph.D. in Immunology from Memorial University of Newfoundland in 2007. Dr. Li is an Instructor in the Department of Immunology at MD Anderson Cancer Center. Her research investigates the molecular regulation of dendritic cells and innate immunity. Her current studies include a focus on improving dendritic cell-based tumor vaccines.

Stephanie S. Watowich received her B.A. in Biology from Carleton College in 1983. She performed cancer research at the University of Chicago from 1983–1985 and obtained her Ph.D. from Northwestern University in 1990. Dr. Watowich conducted postdoctoral studies at the Whitehead Institute of Biomedical Research with Dr. Harvey F. Lodish from 1990–1995, where she discovered the critical importance of erythropoietin receptor dimerization, providing a paradigm for cytokine receptor signal transduction. Dr. Watowich is Professor in Immunology and Co-Director of the Center for Inflammation and Cancer at MD Anderson. Her laboratory investigates the molecular regulation of immunity by cytokines and transcription factors, with a specific focus on the cytokine-activated STATs. Current projects are investigating STAT-mediated regulation of hematopoietic stem cells and mechanisms to enhance anti-tumor immunity.

Highlights

- STAT3 discovery, structure and transcriptional function
- Non-transcriptional activities of STAT3
- STAT3 mutations associate with human immune disorders
- Conditional *Stat3* mouse models reveal important immune functions
- Roles for STAT3 in innate immunity
- Roles for STAT3 in adaptive immunity
- Conclusions and future perspectives

Figure 1. STAT3-immune publication numbers

The number of publications listed in PubMed with the query "STAT3 immune" is shown for each year between 1994 and 2015.

Figure 2. Schematic illustration of a STAT3 dimer bound to DNA

A representation of the major STAT3 domains resolved in the X-ray crystal structure of the STAT3-DNA binding complex is shown. Examples of STAT3 mutations associated with *STAT3* AD-HIES (LOF) or autoimmunity (GOF) are indicated.

Figure 3. Regulation and effects of STAT3 signaling in myelopoiesis

Activation of STAT3 by the G-CSFR during emergency granulopoiesis drives expression of C/EBPβ and c-Myc in granulocytic progenitors, inducing their proliferation and increasing neutrophil production. IFN-γ stimulates SOCS3 expression, which blocks G-CSFR-STAT3 signaling, and promotes PU.1 and IRF8 induction to drive monocyte generation.

Figure 4. Mechanism of the STAT3 anti-inflammatory response

Activation of STAT3 by IL-6R or IL-10R (not shown) inhibits expression of *Ube2n*/Ubc13, thereby restraining TLR4 signal transduction via NF-κB and MAP kinase (not shown) cascades.

Figure 5. STAT3 roles in adaptive immunity

Key functions for STAT3 in precursor B cell generation from CLPs, production of IgG-secreting B cells, and generation of Th17, Tfh and CD8⁺ memory T cells are indicated by orange arrows. Also shown, STAT3 inhibitory activity on Treg generation (blunt-ended orange line). Non-STAT3 functions are indicated by thin black lines.