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STAT3 in CD4⁺ T helper Cell differentiation and Inflammatory Diseases

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Abstract

Jak/STAT pathways influence cell-fate decisions made by differentiating naïve T-cells, regulate the intensity and duration of inflammatory responses and are implicated in pathogenic mechanisms of a number of chronic inflammatory diseases. Among the STATs, the STAT3 protein has emerged as an important determinant of whether the naïve T cell differentiates into regulatory (Treg) or an inflammatory (Th17) T cell lineage. STAT3 also has potent anti-inflammatory effects and regulates critical cellular processes such as, cell growth, apoptosis and transcription of inflammatory genes. Dysregulation of STAT3 pathway has therefore been implicated in the development of chronic inflammatory diseases, as well as, a number of malignant and neurodegenerative diseases. This review focuses on recent findings regarding the role of STAT3 in immunity, with particular emphasis on T cell lineage specification and disease etiology. New insights from animal models of uveitis, multiple sclerosis and inflammatory bowel diseases are discussed as exemplars of critical roles that STAT3 pathways play in inflammatory diseases and on how inhibiting STAT3 can be exploited to mitigate pathogenic autoimmunity.

1. Overview of Jak/STAT Signal transduction pathway

Pathogen recognition by innate immune cells is coupled to secretion of cytokines that inform the adaptive immune system about the nature of the pathogen and instruct naïve T cells to differentiate into the appropriate T cell subtypes required to clear the infection [1]. Thus, naïve T cells are induced to differentiate into Th1, Th2, Th17 and/or regulatory T cells (Treg, Tr1, Th3, TFH) depending on the pathogen eliciting the response [2,3]. Recent studies reveal that IL-6/IL-12 family cytokines (IL-6, IL-12, IL-23, IL-27 and IL-35) play pivotal roles in these lymphocyte cell-fate decisions and their influence on the T cell developmental program is mediated primarily through activation of an evolutionarily conserved family of latent cytoplasmic transcription factors called STATs (signal transducers and activators of transcription) [4-6]. The mammalian STAT family is comprised of 7 members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) that range in size from 750 to 850 amino acids [6].

Upon binding cognate receptors, cytokines and growth factors trigger receptor dimerization/oligomerization that allows rapid transphosphorylation and activation of cytoplasmic kinases [Janus kinase (Jak) 1, Jak2, Jak3 and Tyk2] tethered to some of these receptors. The activated receptor complex provides phosphotyrosine-docking sites for the SH2 domain of STAT

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proteins and following their recruitment, STATs become phosphorylated at a critical tyrosine residue at their C-Terminus [7]. Tyrosine phosphorylation of STATs can also be stimulated following the binding of growth factors to receptor tyrosine kinases such as EGF or PDGF receptors. Tyrosine phosphorylation facilitates formation of parallel STAT homo- or hetero-dimers via reciprocal phosphotyrosine-SH2 interactions in which two SH2 domains are oriented in the same direction [8,9]. Although latent unphosphorylated STAT (U-STAT) proteins also form stable dimers in the cytoplasm, they are structurally distinct from tyrosine-phosphorylated STAT dimers that form after activation [10,11]. Distinct members of the importin family of proteins associate with phosphorylated STATs and transport them into the nucleus where they activate or repress genes that contain cognate GAS (Gamma-interferon activation site) sequences in their promoter [12]. The specific effect on gene transcription derives, in part, from the repertoire of transcriptional coactivators/repressors (p300/CBP, PCAF, GCN5, Cabin 1, mSin3, HDACs) and transcription factors (IRFs, NFAT, NF- κ B, Foxp3, SMAD) recruited to the pre-initiation complex by the STAT member. Upon termination of transcription, STATs are dephosphorylated by nuclear protein tyrosine phosphatases (e.g. TC45, SHP2) and the unphosphorylated dimer is transported back to the cytoplasm by the nuclear export factor, chromosome region maintenance 1 (CRM1) [8]. Besides the initial activation by tyrosine phosphorylation, phosphorylation of a serine residue present at a conserved sequence motif in the STAT C-terminus (e.g. serine 727 in STAT3) provides a mechanism for sustained and maximal activation of the transcriptional activity [13]. An additional layer of complexity of the STAT signaling mechanism is suggested by the presence of GAS sites in STAT1 and STAT3 proximal promoters [14]. Thus, cytokine-induced activation of STAT1 or STAT3 is accompanied by concomitant induction of the expression of unphosphorylated-STAT1 (U-STAT1) or U-STAT3, respectively [14-16]. These U-STATs enter the nucleus by importin-dependent or carrier-independent transport mechanisms that constitutively shuttle U-STATs between the nucleus and cytoplasm, even in unstimulated cells [12,17]. Exciting new data suggest that the U-STAT1 and U-STAT3 associate with IRF-1 and NF- κ B, respectively, and drive expression of repertoires of genes distinct from those induced by the corresponding phosphorylated STAT protein [14-16].

Differential activation of distinct combinations of Jak and STAT proteins, as well as, U-STATs, thus provide a rapid membrane to nucleus mechanism for regulating expression of a diverse array of genes that control host immunity. Several excellent reviews have covered both canonical and non-canonical mechanisms of transcriptional regulation by pSTATs and U-STATs [7,14,15,18,19]. In this article, the emerging role of STAT3 as a major player in the regulation of host immunity will be reviewed, with particular emphasis on exciting new data that put STAT3 at the crossroads of Treg and Th17 differentiation programs. Potential use of STAT3 inhibitors for therapeutic control of inflammatory diseases is also discussed.

2. Structure and function of STAT3

STAT3 is an 89-kDa (770 amino acids) latent cytoplasmic transcription factor characterized by containing each of the following structural protein domains; N-terminal, coiled-coil, DNA binding, linker, SH2 and transcriptional-activation (Figure 1) [7,20,21]. A splice variant of the mammalian *Stat3* with deletion of a 50 nucleotides sequence near the C-terminus, codes for an 80-kDa STAT3 isomer (STAT3 β) that functions as a negative regulator of STAT3 [22]. Similar to other STAT proteins, promoters of STAT3-regulated genes contain the consensus palindromic GAS sequence, TTCN₂₋₄GAA, that defines optimal binding site for each STAT protein. Specificity of the STAT protein for its target genes derives in part, from the characteristic number of non-conserved nucleotides (N) between the palindromic half sites. Thus, in contrast to preference of a canonical N=3 or N=4 nucleotides spacing by STAT1 and STAT6, respectively, STAT3 has a bias for N=2 [20,23].

Death of STAT3-deficient embryos at E6.5-7.5 stage of mouse development revealed the essential role of STAT3 signaling pathway in mammalian physiology [24]. Subsequent studies using Cre-LoxP recombination strategy provided definitive evidence that STAT3 regulates cellular proliferation by inducing cell cycle regulatory genes (e.g. c-myc, pim-1, cyclin D1) and promotes cell survival by upregulating the anti-apoptotic genes, Bcl-2, Bcl-xL, Mcl-1, Fas [25]. However, the role of STAT3 in health and disease is complex as STAT3 regulates an anti-inflammatory transcriptional program that can simultaneously mitigate the development of autoimmune diseases by inhibiting production of proinflammatory genes (IFN- γ , IFN- β , TNF- α , IL-12, CD80, CD86), while promoting establishment of malignancy by suppressing anti-tumor immune responses mediated by cytotoxic T cells [26,27]. Although mechanisms by which STAT3 controls transcription of the diverse array of genes involved in the anti-inflammatory response are poorly understood, recent studies suggest that it is mediated in part, through STAT3-induced inhibition of the ETS family transcriptional repressor, ETV3 (ETS variant gene 3) and the helicase family protein, Strawberry notch homologue 2 (SBNO2) [28,29]. Besides its effects on immunity, proteins that regulate angiogenesis or fibrosis (VEGF and TGF- β) are also targets of STAT3 regulation and importance of STAT3 in cellular physiology is underscored by the wide array of cytokines that utilize STAT3 pathways. These include cytokines that signal through gp130 family receptors (IL-6, CNTF, OSM, LIF, G-CSF), IL-2 γ_c family receptors (IL-2, IL-7, IL-9, IL-13, IL-15 and IL-21), IL-10-related receptors (IL-10, IL-20, IL-22), and receptors with intrinsic tyrosine kinase activity (EGF-R, CSF-1R, PDGF-R) [30-32].

3. STAT Pathways regulate T helper cell lineage Choice

The naïve CD4⁺ T cell that emerges from the thymus is quiescent because genetic loci of key genes that mediate T cell effector functions are silenced by epigenetic mechanisms (CpG-hypermethylation and/or histone deacetylation) [33,34]. However, interaction of naïve lymphocyte with cognate Ag in context of costimulatory signals provided by innate immune cells, induces epigenetic changes that remodel specific cytokine gene loci of the developing/differentiating T cells [33,34]. The cytokine milieu produced by the antigen-presenting innate immune cell influences developmental decisions of the activated naïve T cells to become Th1, Th2, Th17 or T regulatory (Treg, Tr1, Th3) cells and determines the nature of the ensuing immune response (Figure 2) [2,3]. Gene knockout studies have been invaluable in identifying cytokine-induced signaling mechanisms that mediate cell-fate decisions leading to differentiation towards the Th1 or Th2 lineage [4,35]. Commitment to the Th1 lineage requires STAT1- and STAT4-dependent mechanisms that facilitate and/or induce: IFN- γ and T-bet expression; remodeling of IFN- γ chromosomal locus; acquisition of heritable competence for sustained expression of IFN- γ , IL-12R β 2 and IL-18 receptor; and mediate silencing of the *Il4* locus [35]. On the other hand, differentiation towards the Th2 developmental pathway requires STAT6 and GATA3 for induction of epigenetic changes that ensure heritable competence for expressing Th2 cytokine cluster genes (*IL4*, *IL5*, *IL13*) while inhibiting Th1 cytokines and pathways [36]. An important consequence of distinct and non-overlapping patterns of cytokine secretion by Th1 and Th2 cell types is the generation of reciprocal patterns of immunity, such that induction of one type of response antagonizes the induction of the other. Mechanistic details of lineage commitment and maintenance of Th1 and Th2 subtypes have been presented in several excellent reviews [36-38]. The focus here will therefore be on the recently described Th17 and Treg lineages that derive from over-lapping developmental programs.

3.1. STAT3 at the crossroads of Th17 and inducible-Regulatory T cell differentiation programs

During the primary immune response, naïve CD4⁺ T cells can opt for regulatory T cell fate or adopt one of several alternative effector phenotypes. Maintaining appropriate balance between regulatory and effector T cell types ensures effective immunity while avoiding pathologic autoimmunity. Works from several groups suggest that the choice to activate regulatory or inflammatory developmental program is influenced, to a large extent, by the type of PAMPs (pathogen-associated molecule patterns) eliciting the response, antigen-dose, as well as, the strength of T cell-antigen-presenting-cell (APC) interactions. These factors in turn regulate the amounts and types of T cell polarizing cytokines (e.g. IL-12, IL-6, TGF- β) produced in the microenvironment of the differentiating T lymphocytes [39,40]. Of critical importance are TCR-induced transcription factors (NFAT, AP1, NF- κ B) that regulate IL-2 production and CD28-mediated costimulatory signals (NF- κ B, PI3K/AKT) that promote lymphocyte survival and clonal expansion [41-43]. Prolonged stimulation by strong TCR/costimulatory signals induces sustained activation of NFAT, AP1 and NF- κ B while inhibiting expression of Foxp3 (Forkhead box P3 transcription factor). Conversely, signals of low TCR signaling strength or premature termination/blockade of TCR/CD28 signals confer sustained Foxp3 expression and promote unresponsiveness or anergy [39,40]. Thus, increase in NFAT, NF- κ B and AP1 favors development into the rapidly proliferating Th1 and Th17 effector subsets while the Treg phenotype is characterized by increase in Foxp3 expression, impaired calcium mobilization, reduced Ras-Erk-AP1 signaling and lower proliferative capacity [44]. Interestingly, recent reports indicate that NFAT regulates T cell activation and anergy by forming cooperative complexes with either AP1 or Foxp3, respectively [45]. Commitment to effector T cell lineage would therefore be favored by net increases in homeostatic NFAT:AP1 complexes while Treg development and tolerogenic responses would derive from enhanced NFAT:Foxp3 partnering [45]. It is therefore of note, that Th17 or iTreg differentiation program has obligatory requirement for TGF- β 1, a potent inducer of Foxp3. Stimulation of naïve T cells in TGF- β rich environment results in the production of IL-2 and activation of TGF- β 1 signal transduction pathway. Convergence of the STAT5 and SMAD signals on the differentiating cells initiates the development of a Treg/Th17 precursor cell type characterized by expression of Foxp3 and retinoic acid-related orphan receptor (ROR- γ t) transcription factors (see Figure 3). Terminal differentiation of the Treg/Th17 precursor into either the Th17 or iTreg lineage is thought to depend in part on cell-extrinsic factors such as cytokines in the microenvironment that may alter the relative abundance of Foxp3 and ROR- γ t [46,47]. Although stimulation of naïve T cells in TGF- β 1-rich environment is sufficient to induce terminal differentiation into iTreg, production of IL-6 during inflammation and its activation of STAT3, result in upregulation of ROR γ t and skewing of the developmental program towards Th17 lineage [46,48,49]. Thus, the STAT3 pathway is situated at the juncture where Th17 and iTreg developmental programs diverge. Sustained PI3K/Akt/mTOR signaling that occurs in chronic inflammatory conditions has been shown to inhibit TGF- β signaling and Foxp3 expression [50], suggesting that stabilization of NFAT:AP1 at expense of NFAT:Foxp3 complexes may also promote Th17 lineage choice.

3.2. Regulatory T cell Developmental Program

Regulatory T cells are a dedicated subset of T cells that control responses of effector T cells and maintain peripheral tolerance [51,52]. The best-characterized Tregs are the naturally occurring Treg (nTreg) that arise from the thymus and the inducible or activated Treg (iTreg) that are generated in the periphery from conventional CD4⁺CD25⁻Foxp3⁻ T cells during inflammation [51,52]. Both are characterized by constitutive expression of high levels of CD25 and Foxp3. The Foxp3 gene is essential for development and maintenance of Treg cells in the periphery and is induced by TGF- β 1 and IL-2 through SMAD3- and STAT-5-dependent mechanisms (see Figure 3) [53,54]. However, the Foxp3 promoter also contains STAT3

binding motif through which IL-6 can conceivably attenuate its expression in inflammatory conditions [55,56]. Indeed mutual antagonism between Foxp3 and ROR- γ t results in preferential expression of Foxp3 in absence of STAT3 signaling, favoring iTreg development [49]. Stabilization and acquisition of functional competence of the iTreg phenotype derives from upregulation of Treg markers (CTLA-4, CD25, TLR10), reduced capacity for IL-2 secretion and persistence of a state of unresponsiveness to TCR or CD28 signals [57]. The Treg subset also comprise of regulatory T cells that do not express Foxp3 and includes: (i) Type 1 regulatory T cells (Tr1) generated from naive or effector CD4⁺ T cells under chronic stimulation driven by IL-10-secreting immature or tolerogenic DCs [58]; (ii) TGF- β -producing regulatory T cells induced by oral tolerance (Th3) and (iii) IL-21-producing CD4⁺ follicular helper T cells (TFH) that stimulate differentiation of B cells [59]. Despite their current designation as a distinct T cell subset, Tregs do not strictly satisfy criteria for consideration as a separate T helper lineage because they lack a distinct and heritable lineage-specific gene-expression signature. Although Foxp3 is thought of as the master regulator of the Treg developmental program, it is notable that activation of the nTreg or iTreg transcriptional program can proceed independent of Foxp3 [57,60] and in the human, expression of Foxp3 alone is insufficient for regulatory function.

3.3. STAT3 is Essential for Commitment to the Th17 Lineage

The Th17 subset is a distinct T helper lineage characterized by a unique transcriptional program induced by IL-6 and TGF- β 1 and dependent on STAT3 and SMAD signal transduction pathways, respectively. Continued stimulation of the common Treg/Th17 precursor by IL-6 in the lymph node or inflamed peripheral tissues activates STAT3 pathways, resulting in the induction of IL-21 expression. Subsequent binding of IL-21 to its receptor initiates an IL-21/STAT3 autocrine loop that results in the sustained activation of STAT3, induction of ROR- γ t expression and activation of epigenetic changes that cause heritable competence for sustained expression of hallmark cytokines of the Th17 lineage (IL-17A, IL-17F and IL-22) [61] [62]. The Th17 master transcription factors, ROR- γ t and ROR α , induce expression of IL-23 receptor through STAT3-dependent mechanisms, rendering the differentiating cells responsive to IL-23, an innate immune cell cytokine essential for the survival and stabilization of the Th17 phenotype. Activation of STAT3 by each of the critical cytokines (IL-6, IL-21, IL-23) required for establishment of the Th17 lineage, underscore the importance of sustained activation of STAT3 pathway in the Th17 developmental program. Although the role of STAT3 in Th17 development is well established, major gaps still exist in our knowledge of some mechanistic aspects of Th17 development. For example, it is not clear how remodeling of the *IL-17A/IL-17F* loci on murine chromosome 1 and the *IL-22* locus on chromosome 10 are coordinated in order to establish the Th17 lineage. Mechanistic explanation is also lacking for the development of CD4⁺ T cells that express IFN- γ and IL-17 in a number of organ-specific autoimmune diseases [63]. Additional studies are needed to clarify the functional implication for generating two Th17 subsets: The effector Th17 characterized by expression (IL-17⁺, IL-22⁺, CCXL10⁺, CCL2⁺, CCL5⁺) and the Th17 regulatory subset (IL-17⁺, IL-10⁺, IL-26⁺, CCL20⁺, CCL5⁺) [64,65]. These observations beg the question of whether IFN- γ and IL-10 genetic loci are remodeled along with IL-17 and IL-22 loci during Th17 development. If so, does Th17 represents a stable and distinct lineage or merely a prototypic developmental stage that can give rise to each of the well established T cell lineages?

4. Role of STAT3 in CNS inflammatory and neurodegenerative diseases

Aberrant activation of STAT pathways is implicated in a number of human diseases and several STAT members with complementary or antagonistic effects are often activated simultaneously in pathologic conditions. For example, in central nervous system (CNS) inflammatory diseases such as, uveitis and multiple sclerosis, relatively high levels of IFN- γ and IL-6 are detected in

the target tissues and these cytokines mediate their effects through activation of STAT1 and STAT3, respectively. STAT1 is necessary for development of Th1 cells and commonly associated with proinflammatory processes while STAT3 activates an anti-inflammatory program characterized by the upregulation of immunosuppressive cytokines (IL-10, TGF- β 1) that inhibit proinflammatory proteins (IFN- γ , IFN- β , TNF- α , IL-12, chemokines, MHC II, CD80, CD86) [28,66]. It is therefore of note that recent studies in mice suggest paradoxically that Th1 cells may confer protection from chronic inflammatory diseases by antagonizing Th17 effector responses mediated through STAT3 pathways [67]. New insights from mouse models of uveitis and multiple sclerosis are discussed here as exemplar of antagonistic roles played by STAT3 and STAT1 in pathogenic autoimmunity in the retina and brain. The role of STAT3 in neurodegenerative diseases and inflammatory bowel disease are also discussed in context of the unpredictable consequences of constitutive activation of STAT3 in different pathological conditions.

4.1. Loss of STAT3 in CD4⁺ T cells prevents development of CNS inflammatory diseases

Inflammatory processes of CNS tissues, such as the brain and ocular retina, present unique challenges because ultimate goal of eliminating the pathogen must be counterbalanced by the need to limit exuberant inflammatory responses that might compromise functional integrity of these exquisitely delicate immune-privileged tissues [68,69]. This mechanistic dilemma is best illustrated by uveitis, a group of intraocular inflammatory diseases that cause severe visual handicap. This potentially sight-threatening group of idiopathic diseases includes, Behçet's disease, birdshot retinochoroidopathy, Vogt-Koyanagi-Harada, sympathetic ophthalmia, ocular sarcoidosis and may be of infectious or autoimmune etiology [70,71]. Although etiology of non-infectious uveitis is largely unknown, IL-17-expressing T cells are substantially elevated in the blood of patients with active uveitis, suggesting possible involvement of Th17 cells [67]. Surprisingly, significant amounts of Th17 cells are present in the blood of healthy individuals and their levels are substantially elevated following addition of IL-2 to in vitro cultures of human PBMC [67]. Presence of Th17 cells in normal human blood and their expansion by IL-2, suggest that increase in IL-2 that invariably accompany infection may serve as a trigger to expand Th17 cells and promote chronic inflammation. Involvement of Th17 in uveitis has recently been validated in experimental autoimmune uveoretinitis (EAU), a model of uveitis [67]. Analysis of recruitment of uveitogenic T cells from peripheral lymphoid tissues into the retina during EAU, revealed tremendous increase of Th17 cells in the blood or lymph nodes of mice early in the disease process with highest levels detected in the retina at peak of the disease. Their levels decline at latter stages of EAU and the disease is inhibited in mice treated with anti-IL-17 antibodies, suggesting that Th17 cells are positively correlated with EAU pathology. In contrast to Th17 recruitment kinetics in the retina, Th1 levels are low at early stages of EAU and highest levels are detected in the retina at much later stages that coincide with recovery from EAU, suggesting that Th1 cells may participate in mechanisms that mitigate the disease [63]. These studies further suggest that Th17 cells mediate ocular pathology by secreting large amounts of TNF- α in the retina of mice with EAU. Interestingly, IL-27 is constitutively expressed in retina and its expression is upregulated by IFN- γ in retinal cells. As IL-27 inhibits Th17 proliferation, Th1 cells may mitigate uveitis by antagonizing the Th17 phenotype through IFN- γ -mediated induction of IL-27 secretion by the neuroretina [67].

STAT3 is required for commitment of naive T cells towards the Th17 developmental pathway [63,72,73] and consistent with the role of Th17 in etiology of uveitis, mice with targeted deletion of STAT3 in the CD4⁺ T cell compartment (*CD4⁺STAT3^{-/-}*) are resistant to development of EAU [63]. Similarly, *CD4⁺STAT3^{-/-}* mice are resistant to experimental autoimmune encephalomyelitis (EAE), an animal model of human multiple sclerosis, further underscoring requirement of STAT3 pathway in CNS inflammatory diseases [63,74]. Resistance to EAE or

EAU derives in part from marked reduction in the expression of activated $\alpha 4/\beta 1$ integrins and inability of pathogenic Th17 and Th1 cells to enter the eye or brain [63]. In EAU, significant numbers of the IL-17-expressing T-cells also express IFN- γ and these double expressors are absent in *CD4^{STAT3}^{-/-}* mice, raising the intriguing possibility that uveitis maybe mediated not only by Th17 but also by IL-17-expressing Th1 cells. Requirement of STAT3 for generation of Th17 and trafficking of Th17 and Th1 cells into the CNS, suggest that the STAT3 pathway is a potential therapeutic target that maybe used to prevent or mitigate uveitis or multiple sclerosis.

4.2. Inflammatory bowel disease (IBD)

Th17 cells and activation of STAT3 pathway are also implicated in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), two major forms of inflammatory bowel diseases (IBD) in humans [75,76]. However, their exact role in IBD is controversial. In support for role of STAT3 and Th17 cells in IBD are the findings that the degree of gut tissue inflammation correlates with the level of pSTAT3 in histological sections of IBD patients [75,77]. Moreover, the progressive damage to the gut is characterized by aberrant inflammatory response to components of the bacterial microflora and Th17 cells are thought to contribute in the destruction of gut tissues by inducing secretion of extracellular matrix-degrading enzymes, MIP-3 α and IL-21. Autocrine secretion of IL-21 that perpetuates a cycle of elevated IL-21 secretion and sustained STAT3 activation in the gut plays important roles in exacerbating the disease [77]. In addition, pSTAT3 enhances survival of the pathogenic Th17 cells by upregulating Bcl-2, Bcl-xL, and Mcl-1 genes [25] and may thereby contribute in maintaining the chronic inflammatory process. However, the role of IL-17 in the development of intestinal inflammation has been disputed because the closely related IL-17 isoforms, IL-17 and IL-17F, appear to exert diametrically opposite effects on the development of colitis [49,78]. Blocking IL-17 activity in vivo using anti-IL17 neutralizing mAb exacerbated dextran sulfate sodium sulfate (DSS)-induced colitis, suggesting an inhibitory role for IL-17 in DSS-colitis [79]. In addition, IL-17 knockout mice developed more severe disease and IL-17F deficiency reduces DSS-induced colitis [49]. Thus, there is a growing consensus that IL-17 may play a protective role, while IL-17F may exacerbate the intestinal inflammation. Implicit in this viewpoint is the possibility that other cytokines produced by Th17 cells may also have a role in amelioration or pathogenesis of IBD. In fact, IL-22 suppresses Th2-mediated colitis through STAT3-dependent production of IL-10 and SOCS3 (suppressors of cytokine signaling) [75,80] and production of IL-22 by innate (natural killer cells) and adaptive (Th17) immune cells confers protection from pathologic gut inflammation [81]. Furthermore, mice with targeted deletion of STAT3 in macrophages develop chronic enterocolitis due to impaired production of IL-10, suggesting that STAT3-mediated activation of innate responses contributes to the suppression of colitis [82]. Collectively, these studies suggest two distinct roles for STAT3 and Th17 cells in IBD: Whereas, STAT3-mediates pathologic immune responses that exacerbate colitis by inducing and enhancing survival of IL-17F-expressing Th17 cells, production of IL-17 and IL-22 by the Th17 and innate immune cells mitigates pathogenic responses to bacterial flora. Thus, targeting STAT3 as therapy for IBD should be attempted with caution

4.3. Pathological consequences of aberrant regulation of STAT3 activity

Myriad of pathways regulated directly or indirectly by STAT3 necessitates stringent regulation of pSTAT3 and U-STAT3 activities. A number of endogenous negative regulators attenuate STAT activity by directly targeting the phosphorylated STAT protein and these include the SH2-domain containing tyrosine phosphatase family (SHP-1 and SHP-2) and protein inhibitors of activated STATs (PIAS) family proteins (see Figure 4). STAT pathways are also under classical feedback regulation by SOCS proteins, a family of intracellular proteins that are rapidly induced by STATs in response to cytokine/growth factor stimulation [83,84]. For example, SOCS3 expression is induced by pSTAT3 in response to cytokines that signal through

gp130-related cytokine receptors and it attenuates or terminating the STAT3 signal by targeting the receptor and its associated JAK kinases for degradation (Figure 4). An essential attribute of SOCS proteins is their relatively short half-lives which ensures that their negative regulatory effects are transient. Thus, unabated stimulation of STAT pathways resulting in persistent activation of SOCS proteins has been observed in many pathological conditions. In the neuroretina, neurons are protected from apoptosis by neurotrophic factors that mediate their functions through STAT3 pathways (CNTF, LIF, VEGF) and activation of STAT3 is a common physiologic response of neuroretinal cells to cellular stress that induce apoptotic or necrotic death of photoreceptors [85-88]. In a recent study, chronic intraocular inflammation, light-induced retina damage, oxidative stress and metabolic stress due to type 1 diabetes were found to persistently activate SOCS1 and SOCS3 expression, resulting in the induction of insulin resistance and retinal degeneration [88]. It is now widely accepted that unrestrained neuroinflammatory responses underlie neuronal or photoreceptor cell deficits that precede neurodegenerative changes in multiple sclerosis, uveitis, Alzheimer's disease or age-related macular degeneration and these observations in the neuroretina suggest that STAT3/SOCS3 dysregulation may be at the crossroads of inflammation and idiopathic retinopathies. In this context, it is interesting to note that targeted expression of a proinflammatory cytokine (IFN- γ) in the eye of transgenic rats induced constitutive expression of SOCS proteins in the neuroretina and retinal degenerative changes similar to human retinal dystrophies [89,90]. Role of STAT3/SOCS3 dysregulation in disease is not restricted to neuroinflammatory diseases: hypermethylation of CpG islands of the SOCS1 or SOCS3 gene resulting in loss of feedback regulation of STAT3 signaling has also been implicated in lung cancer [91], breast and ovarian cancer [92], squamous cell carcinoma [93], glioblastoma, and hepatocellular carcinoma [94].

5. Concluding remarks

It is important to note that during uveitis or encephalitis, all 4 major T cell subsets are detected in the retina, brain or spinal cord and presence of any T cell type in the eye is undesirable because of interference with vision and potential damage to neurons by inflammatory cytokines. A major therapeutic goal is therefore to prevent any T cell type from entering the CNS or limit their expansion in these immune privileged sites. Data indicating that targeted deletion of STAT3 in CD4⁺ T cells limits access of T cells into the CNS and prevents development EAU or EAE, suggests that STAT3 is a potential target for modulating CNS inflammatory diseases. Recent successes in use of synthetic STAT3 inhibitors to inhibit EAU (unpublished data) may usher new avenues for treating these intractable diseases.

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Figure 1.

Structure of STAT3 protein. Similar to other members of the STAT family, STAT3 is comprised of six domains: N-terminal domain, coiled coil domain, DNA binding domain, linker domain, SH2 domain and Tran activation domain.

Figure 2.

Outline of T-helper-cell differentiation. During the initial activation of CD4⁺ lymphocyte, the antigen presenting dendritic cells secrete a variety of cytokines that instruct the naïve T cell to activate one of several alternative T helper cell developmental pathways leading to Th1, Th2, Th17 or Treg lineage. Each T helper phenotype produces its signature cytokines that mediate its distinct immunoregulatory functions.

Figure 3.

STAT3 at the nexus of Th17/Treg developmental programs. Differentiation into Th17 or inducible regulatory T cell (iTreg) lineage has an obligatory requirement for signals provided by TGF-β. Shortly after the activation of naive CD4⁺ T cells, convergence of IL-2 and TGF-β1 signals generates the common Treg/Th17 precursor characterized by expression of Foxp3 and ROR-γt. Further stimulation by TGF-β1 favors iTreg differentiation while maturation of the Treg/Th17 precursor in inflammatory niche with high levels of IL-6, inhibits Foxp3 and skews development towards Th17 phenotype. The Th17 master transcription factors, RORγt and RORα, induce expression of IL-23 receptor through STAT3-dependent mechanisms, rendering the differentiating Th17 cells responsive to IL-23, a cytokine essential for survival

and stabilization of the Th17 phenotype. Sustained signaling through the IL-23 receptor might be required for the establishment of terminally differentiated Th17 effector phenotype.

Figure 4.

Regulation of STAT3 signal transduction pathway. Cytokine binding induces receptor oligomerization that facilitate cross-phosphorylation and activation of the receptor-associated JAK kinases. Recruitment of STAT3 to activated gp130 receptors in response to cytokines result in their activation, dimerization and translocate into the nucleus to induce the expression of cytokine responsive genes including SOCS3 and to a lesser extent, SOCS1. The SOCS proteins inhibit or terminate JAK/STAT signals by binding to the activation loop tyrosine residues of JAKs and/or the tyrosine-phosphorylated cytokine receptors and target them for degradation by the proteosome. The STAT3 signal can also be attenuated by PIAS3, a member of the protein inhibitors of activated STATs (PIAS) family of proteins. PIAS3 binds selectively to activated STAT3 dimers and blocks their ability to bind DNA and activate transcription.