CHAPTER 10

Targeting Th17 and Treg Signaling Pathways in Autoimmunity

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Contents

1. INTRODUCTION

Chronic inflammatory and autoimmune disorders encompass a broad set of individual clinical diseases that share underlying pathophysiologic features. These diseases are increasingly common, represent a significant unmet clinical need, and have galvanized substantial interest and investment within the biopharmaceutical industry. Although some idiopathic inflammatory disorders are linked to innate immune dysregulation, the majority of chronic inflammatory and autoimmune diseases are driven by misguided or over-aggressive T cell responses. Accordingly, a

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comprehensive understanding of how T cells, particularly $CD4^+$ T helper (Th) cells, develop under both normal and pathologic conditions is instrumental in guiding new drug discovery.

T cells are of hematopoietic lineage; like all blood cells they develop from pluripotent progenitors resident to the marrow of long bones [1]. T cell precursors migrate from bone marrow to the thymus where they pass through a series of maturation stages to form a mature T cell that ultimately circulates through blood and lymphatic vessels [2]. In healthy individuals, the end result of T cell ontogeny is an army of mature naïve T cells, each expressing an individualized T cell antigen receptor (TCR) that will recognize unique danger signals in the form of microbe-derived protein fragments (*i.e.*, antigens), while ignoring those from host tissues or commensal microorganisms. However, even in healthy individuals, a small number of self-reactive T cells can, and do, escape the thymus [3]. In response, nature has evolved a failsafe mechanism to ensure that T cell tolerance toward host tissues is maintained, involving the parallel development of T regulatory (Treg) cells. Treg cells are distinguished from conventional naïve T cells by their constitutive expression of the transcription factor Forkhead box, winged-helix protein 3 (Foxp3); they preferentially recognize self-antigens and act to dampen the activation of local T cells through suppressive mechanisms that remain poorly elucidated [4–6]. Current paradigms suggest that the balance between conventional naïve T cell activation (and subsequent differentiation into effector subsets, see below) and Treg-mediated immune suppression controls whether immune responses are ultimately protective, ineffective, or pathogenic [7,8]. Indeed, early clinical results utilizing Treg cellular therapy supports the notion that increasing Treg numbers in autoimmune patients can support tolerance [9].

1.1. Th17 pathway

Conventional naïve T cells become activated in response to cognate antigen presented in the context of MHC class II molecules on the surface of professional antigen-presenting cells (APC), such as B cells, monocytes, macrophages, and dendritic cells. In addition to TCR signal transduction, APC also engage a number of co-receptors on the surface of T cells, which can either act to enhance or inhibit T cell activation [10]. Further, APC can produce an array of cytokines that act as tertiary signals to T cells, instructing them to differentiate into specialized effector T cell subsets (Th cells), which in turn orchestrate specific immune reactions aimed at clearing individual classes of pathogens. Originally, Th cell differentiation was thought to be bimodal, either resulting in T helper type 1 (Th1) or T helper type 2 (Th2) cell development. Whereas Th1 cells produce gamma-interferon (IFNγ) and activate phagocytic and cytolytic immunity

against intracellular pathogens (*e.g.*, viruses), Th2 cells produce IL-4, IL-5, and IL-13 to induce humoral immunity against extracellular parasites [11]. However, recent advances in T cell biology have expanded the list of potential T cell subsets (see Figure 1). In particular, Th17 cells, which express IL-17A (*i.e.*, IL-17), IL-17F, and IL-22, have been implicated in mucosal immunity directed against fungal pathogens and some species of bacteria. These cells are also broadly implicated in the pathogenesis of most common autoimmune and chronic inflammatory disorders, including rheumatoid arthritis (RA), multiple sclerosis (MS), and inflammatory bowel diseases (IBD) [12,13].

The cytokines responsible for directing naïve T cell differentiation into Th17 cells include transforming growth factor β (TGF-β) and the acute phase protein IL-6 [14–16]. This combination of cytokines potently activates signal transducer activator of transcription (Stat)-3, which subsequently promotes expression of the retinoic acid-related orphan nuclear receptor RORγt (RORC in humans) [15–17]. Stat3 and RORγt subsequently function in a synergistic fashion to activate expression of IL-17. In addition, IL-23, another Stat3-activating cytokine, has been shown to act on developing Th17 cells to enforce IL-17 expression and stabilize the

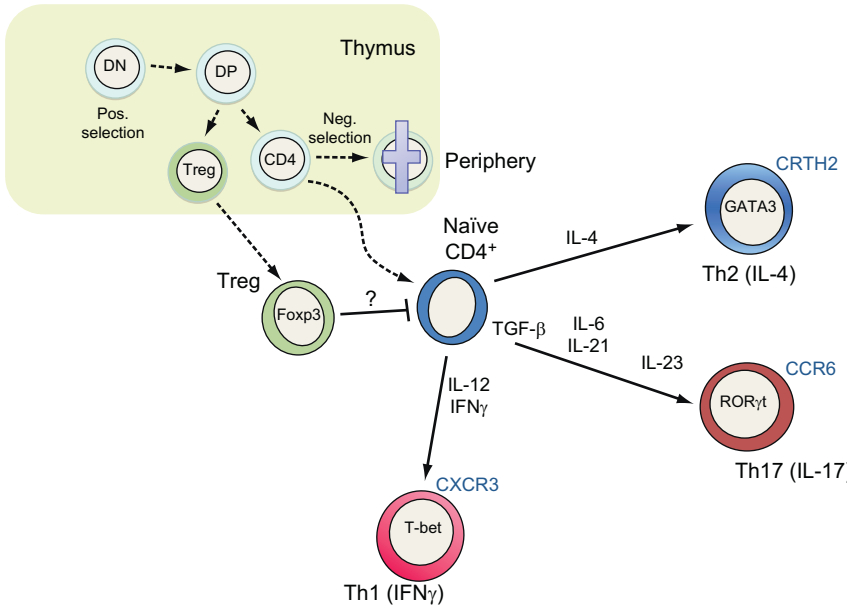


Figure 1 Model of T cell development and differentiation. Naïve T cells and Tregs develop in the thymus. Naïve T cells become activated by antigens in the periphery and can differentiate into one of three effector linages (*e.g.*, Th1, Th2, Th17). Regulatory T cells block the activation of bystander naïve T cells.

Th17 lineage [18]. Given the broad role of Th17 cells reported in immunoinflammation, inhibiting Th17 cell development and/or function has profound therapeutic implications for autoimmune and chronic inflammatory indications.

At least two distinct strategies can be envisaged to target Th17-mediated inflammation: (1) blocking Th17 effector function and (2) blocking Th17 differentiation. By either strategy, targeting Th17 cells specifically represents a significant advance over current clinical modalities that act more broadly to cripple the immune system (*i.e.*, immunosuppressants, cyclosporine A, rapamycin). The drug discovery/development efforts of both these strategies will be discussed in the next section.

1.2. Treg pathway and immune homeostasis

Evidence from both autoimmune mouse models and human patients reveal that Treg cells are necessary to prevent spontaneous autoimmunity throughout life [19,20]. However, Treg cells do not necessarily distinguish among the types of T cell responses they inhibit. Recent data clearly indicates that Treg cells can also regulate immune responses to pathogens and developing tumors [21–23]. In fact, solid tumors may even actively recruit Treg cells as a means to preventing immunosurveillance [24]. Although human Treg biology is still in its infancy, we highlight some recent clinical advances that may modulate Treg function.

2. CURRENT TARGETS AND MOLECULES IN DEVELOPMENT

2.1. Th17 effector function

Several approaches have been taken to block Th17 cell cytokines. Current molecules and their development statuses are detailed below.

2.1.1. IL-17/IL-17 receptor antibodies

IL-17 and IL-17F are known to induce local cytokine and chemokine production, resulting in tissue inflammation characterized by neutrophil recruitment. IL-17 has been shown to play a key role in preclinical animal models including collagen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE) [25–29].

Secukinumab (AIN457), a monoclonal antibody (mAb) that neutralizes IL-17, is being evaluated for the treatment of uveitis, psoriasis, and other inflammatory conditions. In a Phase 3 study, AIN457 did not meet its primary endpoint in the treatment of non-infectious uveitis in patients with Behcets disease [30,31]. AIN457 is currently being evaluated in other uveitis studies [32]. Proof of concept has been shown in other

inflammatory indications including psoriasis, RA, and ankylosing spondylitis. In psoriasis patients, AIN457 treatment showed greater benefit than placebo at all time points up to week 12, as measured by PASI50 (Psoriasis Area and Severity Index 50), reductions of histomorphological signs of acanthosis and epidermal hyperplasia, and changes in gene expression of markers of the IL-17A pathway [33]. In the RA trial, patients achieved ACR20 (American College of Rheumatology 20) response rates (50% for AIN457 and 31% for placebo) by week 4 ($P = 0.13$) which were maintained at week 16 (54% *vs*. 31%; $P = 0.08$). The 28-joint disease activity scores (DAS28) and C-reactive protein (CRP) values significantly decreased over time [33]. In a Phase 2 ankylosing spondylitis trial, AIN457 induced significantly higher ASAS20 (Assessment of SpondyloArthritis international Society 20) responses than placebo at week 6 meeting the primary endpoint [34].

Other anti-IL-17 humanized mAbs under clinical development include LY2439821 for the treatment of RA and psoriasis, RG-4934 for the treatment of RA, RO-5310074 for the treatment of psoriatic arthritis (PsA), and MEDI-571 for the treatment of RA [35–38]. AMG 827, a fully human mAb that binds to the IL-17 receptor and blocks its signaling, is currently being investigated as a treatment for a variety of inflammatory disorders including RA and psoriasis [39,40].

2.2. Th17 differentiation

Current understanding of Th17 cell differentiation and maintenance has indicated several points of therapeutic intervention. These include blocking of critical cytokines IL-6, IL-21, and IL-23 and their receptors, blocking the JAK/STAT pathway and antagonizing transcription factors such as RORγt and the aryl hydrocarbon receptor (AhR).

2.2.1. IL-6/IL-6 receptor antibodies

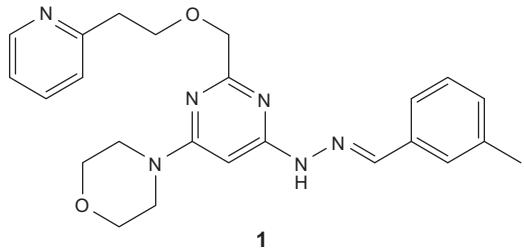
As discussed above, IL-6 is indispensable for the differentiation of Th17 cells from naïve precursors; it also inhibits TGF-β-induced expression of Foxp3 [16,41]. Stimulation by IL-6 in the lymph node or inflamed peripheral tissues activates JAK/STAT pathway, resulting in Stat3 activation, RORγt (and RORα) expression, and subsequent *trans*-activation of both IL-21 and the IL-23 receptor (IL-23R). Autocrine signaling through IL-21/IL-21R and through IL-23 further stabilizes Stat3 activity resulting in epigenetic modifications at the *Il17a/f* locus that allow for sustained inflammatory cytokine production [42].

Tocilizumab, a recombinant humanized mAb against IL-6 receptor (IL-6R), has been approved for the treatment of RA and Castleman disease. The antibody is currently being evaluated in Phase 3 trials for ankylosing spondylitis [43]. SAR-153191 (REGN-88), an IL-6R antibody,

is in Phase 3 trials for RA and ankylosing spondylitis [44]. Olokizumab, CNTO-136, and ALD-518 are anti-IL-6 antibodies being evaluated in Phase 2 trials for RA [45–47]. CNTO-136 is also in Phase 2 trials for systemic lupus erythematosus (SLE) and lupus nephritis [48].

2.2.2. IL-23 and IL-12/23 antibodies

IL-23 is a heterodimeric cytokine produced by activated APC. It comprises IL-23p19 and IL-12p40, and signaling through the IL-23R is essential for the survival and stabilization of the Th17 phenotype. IL-23 has been implicated in several inflammatory conditions such as colitis, gastritis, arthritis, and psoriasis [49–53]. Ustekinumab, a humanized antibody targeting the p40 subunit of IL-12 and IL-23, was approved recently for the treatment of psoriasis. The antibody is in Phase 3 trials for PsA and Phase 2 trials for Crohn's disease [54,55]. SCH-90222, an anti-IL-23 antibody, is in development for psoriasis (Phase 2) [56]. An orally bioavailable small molecule inhibitor of IL-12 and IL-23 production, STA-5326, **1**, is also under development for psoriasis and Crohn's disease [57]. STA-5326 inhibits c-Rel translocation which results in inhibition of the expression of genes encoding the p40 subunit present in both IL-12 and IL-23 [58].



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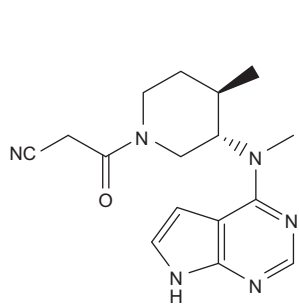
2.2.3. JAK/STAT pathway inhibitors

IL-6, IL-21, and IL-23 all regulate Th17 differentiation through their activation of Stat3. Stat3 activation downstream of these receptors is mediated by receptor associated Janus kinases (JAKs), which include JAK1, JAK2, JAK3, and TYK2. Several small molecule modulators targeting the JAK/STAT pathway have been developed that affect Th17 function and have anti-inflammatory activity; some examples are discussed below.

CP-690550, **2**, is a pan JAK inhibitor with low nanomolar potency against JAK1, JAK2, and JAK3, but with functional selectivity for JAK1/3 *versus* JAK2 in cellular assays [59,60]. CP-690550 has shown preclinical efficacy in mouse CIA and rat adjuvant-induced arthritis models [59,60]. CP-690550 is currently in Phase 3 trials for RA and Phase 2 trials for prevention of acute (renal) allograft rejection, psoriasis (oral and topical),

Crohn's disease, ulcerative colitis, and dry eye disease (topical). In the ORAL Sync Phase 3 study, in moderate-to-severe RA, CP-690550 met its primary endpoints showing statistically significant changes *versus* placebo in reducing signs and symptoms of RA, based on ACR20 response rates at 6 months and improved physical function [61,62].

INCB018424, **3**, is a selective small molecule inhibitor of JAK1 and JAK2 that potently inhibits cytokine-induced JAK signaling and function in lymphocytes and keratinocytes [63]. In an open label subtotal inunction study in 25 patients with plaque psoriasis, transcriptional changes in biopsies at baseline and following 28 days of topical INCB018424 treatment were consistent with decreased Th1 and Th17 lymphocyte activation, decreased epidermal hyperplasia and dendritic cell activation. In a subsequent Phase 2b study, the primary endpoint of total lesion score for all dose groups was decreased greater than two-fold over vehicle control at day 84 [63]. INCB028050, a selective orally bioavailable JAK1/JAK2 inhibitor, is currently under clinical evaluation for the treatment of RA [64].



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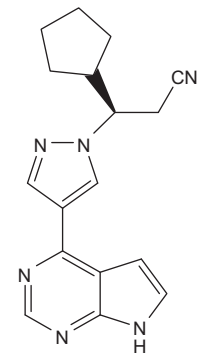

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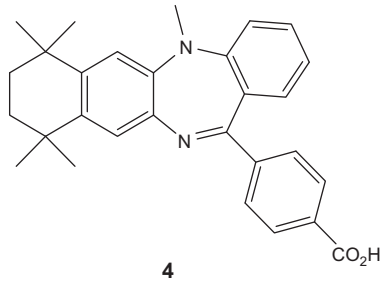
2.2.4. Inhibitors of transcription factors

Activation of STAT3 by each of the critical cytokines (IL-6, IL-21, IL-23) results in the induction of RORγt and RORα, which subsequently leads to expression of IL-17. Forced overexpression of RORγt in human naive T cells induces a Th17-like phenotype, by inducing IL-17A, IL-17F, IL-26, and CCR6 expression and downregulating IFN-γ secretion [15,65,66]. *In vivo*, RORγt-deficient mice are protected in an EAE model, show reduced susceptibility to allergen-induced airway inflammation, and are protected against crescentic glomerulonephritis [15,67,68]. In addition, it also has been shown that RORγt-deficient T cells do not induce colitis when adoptively transferred [69]. There have been very few reports in the

literature that disclose RORγt inhibitors. Jetten *et al.* have shown that selective LXXLL peptides (*e.g.*, VLVEHPILGGLLSTRVDSS) bind to the ligand binding domain of RORγt and antagonize RORγt-mediated transcriptional activation [70]. It has been reported that carboxylic acid-containing compounds (*e.g.*, LE 135, **4**) structurally related to all *trans* retinoic acid (ATRA) are RORγt inhibitors which reduced IL-17 production from activated human peripheral blood mononuclear cells in a dose-dependent manner [71]. Recently, Huh *et al*. reported that digoxin, **5**, a cardiac glycoside, and two synthetic derivatives selectively inhibited RORγt activity and suppressed mouse and human Th17 differentiation. Treatment with **5** delayed onset and reduced severity of disease in a mouse EAE model [72].



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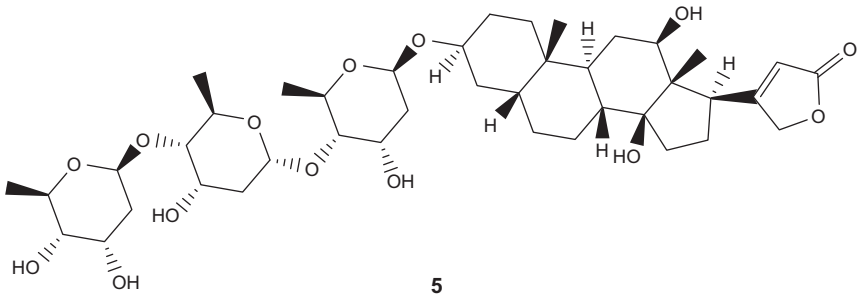

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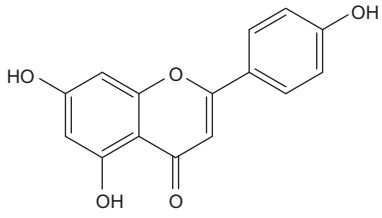
AhR, a ligand-activated transcription factor, has been shown to regulate Th17-cell development and Treg differentiation in mice [73,74]. AhR expression in $CD4^+$ T cells in mice was found to be restricted to the Th17 cell subset and is essential for IL-22 production. AhR-deficient mice develop less severe disease in an EAE model. Studies indicate that AhR may also be involved in the expression of the anti-inflammatory T cell cytokine IL-10 during T cell differentiation [75,76]. Several flavonoids including apigenin, **6**, naringenin, **7**, and CH-223191, **8**, function as AhR antagonists which may be useful in the treatment of autoimmune diseases [77].



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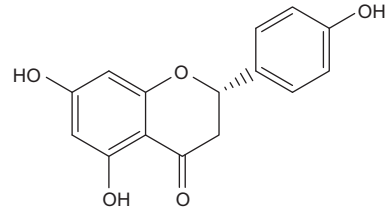

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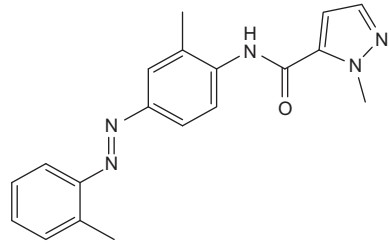

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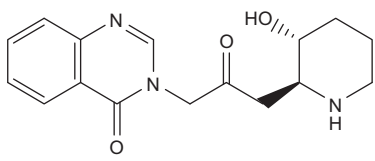
2.2.5. Halofuginone and the amino acid starvation response

Halofuginone, **9**, is a synthetic derivative of febrifugine, **10**, a naturally occurring alkaloid found in the root of hydrangea plants. Halofuginone has been reported to be a potent and selective inhibitor of Th17 differentiation which functions by inducing a state of nutritional stress known as the amino acid starvation response. Treatment of naïve T cells with **9** was found to block Th17 differentiation and concomitantly increase Foxp3 expression without impacting cell proliferation, or Th1 or Th2 differentiation. Administration of **9** to mice selectively reduced both Th17 differentiation and the development of Th17-driven EAE. In a second EAE model driven entirely by IFNγ-producing Th1 cells, **9** did not prevent disease onset or severity [78]. The direct cellular target of **9** remains unknown.



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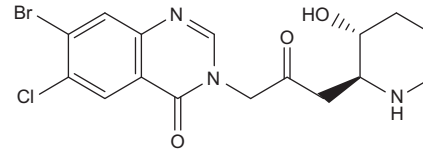

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2.3. Treg biologics

2.3.1. Anti-CD3 antibodies

Anti-CD3 antibodies act as immunosuppressants, both by reducing the number of effector T cells and inducing the development of adaptive Tregs, although the underlying mechanism is not fully understood [79]. Muromonab-CD3 (a mouse mAb against human CD3) was approved for the prevention of renal allograft rejection, but an important side effect is CRS (cytokine release syndrome) [80,81]. Two humanized FcR nonbinding anti-CD3 antibodies (teplizumab and otelixizumab) have since been developed. In recent Phase 3 studies in patients with recent-onset Type 1 diabetes mellitus (T1DM), both teplizumab and otelixizumab failed to meet the primary end points [82,83]. Trials with otelixizumab in adolescents and adults with newly diagnosed T1DM, RA, and thyroid eye disease are ongoing [84–89]. A Phase 2 trial is currently underway to evaluate if teplizumab can help prevent or delay the onset of T1DM in relatives at high risk of developing the disease [90].

2.3.2. CTLA-4-Ig fusion protein

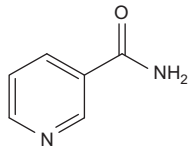
CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4) is a cell-surface molecule that binds CD80 and CD86, resulting in an inhibitory signal that leads to suppression of T cell proliferation. Treg-specific CTLA-4-deficient mice spontaneously develop systemic lymphoproliferation, fatal T cell-mediated autoimmune disease, hyperproduction of immunoglobulin E, and enhanced tumor immunity [91].

Abatacept (ORENCIA®), a fully human fusion protein that binds to CD80/CD86 with high affinity, has been approved for RA and juvenile idiopathic arthritis [92]. Trials to evaluate abatacept in Crohn's disease, ulcerative colitis, and in non-life-threatening SLE failed to meet their primary endpoints [93–96]. Efficacy was observed in the treatment of 170 patients with PsA with 48% of patients achieving ACR20 for abatacept *versus* 19% for placebo ($P = 0.006$) [97]. Additional trials in prevention of GVHD and in the treatment of lupus nephritis are ongoing [98].

2.4. Emerging targets influencing Treg function

Epigenetic regulation and posttranslational modification of Foxp3 in Tregs have been studied by several groups. Loosdregt *et al.* reported that Foxp3 acetylation is regulated by histone acetyltransferase p300 and histone deacetylase SIRT1 [99]. *Ex vivo* treatment of $CD4^+$ T cells with SIRT1 inhibitor nicotinamide, **11**, resulted in increased Foxp3 levels and increased suppressive activity. An evolutionarily conserved CpG-rich element within the Foxp3 locus was identified by Huehn *et al.* that was

selectively demethylated in natural Tregs (nTregs), but not in conventional T cells or in *in vitro* generated iTregs [100]. The methylation status of this Treg-specific demethylated region (TSDR) can be manipulated by inhibitors of DNA methyltransferase 1 (DNMT1), such as 5-azacytidine **12**. Huehn *et al.* reported that **12** promoted a more stable Foxp3 expression [101]. Hancock *et al.* have reported that Tregs isolated from HDAC9-deficient mice were more abundant and displayed increased suppressive function *in vitro* and *in vivo*; these cells also showed enhanced expression of Foxp3, CTLA-4, and GITR (glucocorticoid-induced TNFR-related protein), as well as increased acetylation of Foxp3 [102]. The HDAC9-deficient mice are also reported to be resistant to DSS-induced colitis [103]. Similar effects have been reported with Trichostatin A (**13**), a pan-HDAC inhibitor, on Treg numbers and in prevention of DSS-induced colitis [102,103].



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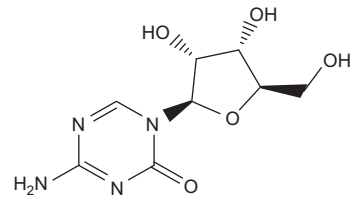

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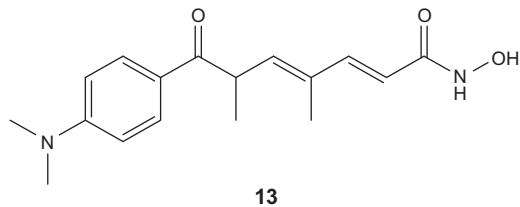

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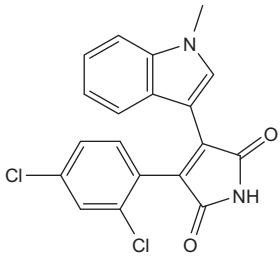
Two different kinases have been reported to modulate Treg function. Glycogen synthase kinase-3 (GSK-3β) regulates β-catenin, which has been shown to prolong Treg survival [104]. A GSK-3β inhibitor (SB216763, **14**) was reported to increase Treg suppressive activity and prolong Foxp3 levels [105]. *In vivo*, SB216763 treatment afforded a modest effect in prolonging islet survival in an allotransplant mouse model. Zanin-Zhorov *et al.* have reported that treatment with Protein Kinase C-theta (PKC-θ) inhibitor C20 (**15**) protected Tregs from inactivation by TNF-α, enhanced suppressive function of defective Tregs from RA patients, and enhanced the protective capabilities of Tregs in a T cell induced colitis model [106].



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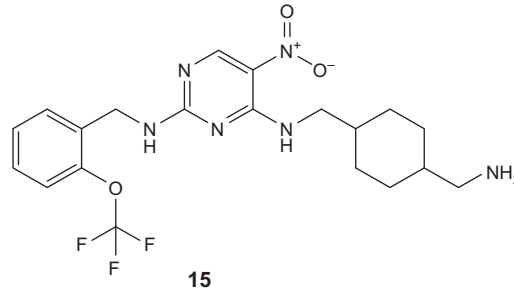

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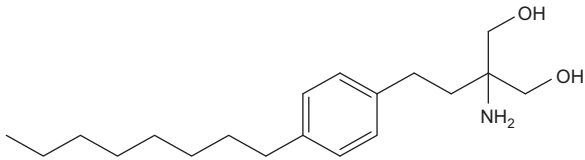
Recent studies have shown that fingolimod, **16**, an S1P receptor modulator, increases the functional activity of Tregs [107]. In a mouse model of colitis, treatment with fingolimod resulted in upregulation of Foxp3, IL-10, TGF-β, and CTLA-4, and it significantly suppressed the development of disease [108]. There are several reports that TLR ligands can modulate Treg function: TLR7 agonists imiquimod, **17**; gardiquimod, **18**; and flagellin (a TLR5 ligand) have been reported to enhance Treg suppressive function [109,110].



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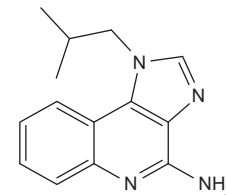

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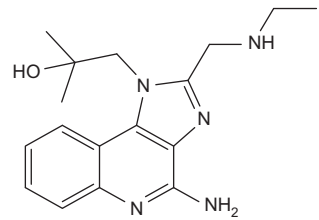

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3. CONCLUSIONS

T cell-driven autoimmune disorders continue to present a significant unmet clinical need. Advances in our understanding of T cell activation, differentiation, and regulation have yielded novel approaches to

specifically dampen pathogenic immune reactions without creating potentially dangerous states of general immune suppression. As discussed throughout, specific modulation of Th17 and Treg cells affords broad therapeutic promise for the treatment of autoimmune and chronic inflammatory conditions. Clinical results from the current biopharmaceutical therapies will provide further validation for such targeted pathways. Even though there are relatively few reports of small molecule modulators of Th17 and Treg cell function, several targets have been identified providing new opportunities for small molecule drug discovery.

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