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Small-molecule control of cytokine function: new opportunities for treating immune disorders

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Abstract

Manipulating cytokine function with protein-based drugs has proven effective for treating a wide variety of autoimmune and auto-inflammatory disorders. However, the limited ability of protein-based drugs to modulate intracellular targets, including many implicated by studies of the genetics and physiology of these diseases, and to coordinately neutralize redundant inflammatory cytokines, suggest an important and complementary role for small molecules in immunomodulatory drug development. The recent clinical approval of Janus kinase and phosphodiesterase inhibitors, along with emerging evidence from other compound classes, firmly establish small molecules as effective tools for modulating therapeutically relevant proteins that give rise to aberrant cytokine signaling or mediate its downstream consequences.

Introduction

The incidence of autoimmune and auto-inflammatory disorders is rapidly increasing in developed countries [1]. Addressing this clinical need will require continued innovation in immunomodulatory drug development. Data from many sources, including analysis of how human genetic variation affects disease susceptibility, implicate aberrant cytokine production and signaling in the pathophysiology of these disorders (see Box 1 for background on the application of disease genetics to drug discovery). For example, mutations in the cellular machinery that processes the inflammatory cytokine interleukin-1β (IL-1β) to its mature form cause hereditary auto-inflammatory diseases known as cryopyrin disorders (Figure 1a) [2]. Protein therapies inhibiting IL-1β (canakinumab; rilonacept) or its receptor (anakinra) are used to treat cryopyrin disorders, as well as immune disorders with more complex

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etiologies, including gout, type-2 diabetes, rheumatoid arthritis (RA) and chronic granulomatous disease [3,4]. The clinical success of biopharmaceuticals targeting IL-1β or other cytokines (TNF-α, IL-6, IL-12/23) derives from their ability to disrupt protein–protein interactions with exquisite selectivity and predictable, long-lasting pharmacology [5].

Despite this success, several limitations of biopharmaceuticals hamper therapeutic manipulation of cytokine networks. Most notably, protein-based therapies are unable to regulate intracellular proteins, including many potential targets identified by disease genetics and recent studies of the mechanisms that regulate immune cell development and function [6-8]. Also, while systemic administration of blocking antibodies or decoy receptors can effectively neutralize individual cytokines in circulation, these effects can be undermined by functional redundancy among inflammatory cytokines or limited delivery of protein-based reagents to mucosal tissues [5,9]. Finally, biopharmaceuticals are expensive to produce and lack oral availability, often necessitating administration by specialists.

Small molecules constitute a complementary approach to immunomodulatory drug development by enabling modulation of intracellular proteins that give rise to aberrant cytokine signaling or mediate its downstream consequences. Endogenous small molecules such as eicosanoids have long been recognized to play a key role in controlling tissue-specific inflammation [10], and the impact of metabolites made by commensal microbes on cytokine-producing cells is increasingly clear [11-13]. Moreover, drugs modulating intracellular signaling proteins (rapamycin targeting mTOR [14]; FK506 and cyclosporine A targeting calcineurin [15]) were among the first immunomodulatory therapies approved by the FDA. More recently, small molecules have been discovered that modulate cytokine function through a range of mechanisms-of-action (Table 1). These successes establish small molecules as a complementary alternative to protein-based therapies for regulating cytokine networks. Here, we review recent findings that motivate discovery of small molecules targeting kinases, other classes of signaling proteins and transcriptional regulators implicated in aberrant cytokine signaling by the genetics and physiology of autoimmune/auto-inflammatory disorders.

Regulation of immune cell signaling with kinase inhibitors

Small molecules have been used successfully to manipulate immune cell signaling at several levels. Prostanoid receptor agonists are being explored as IBD therapies [10], whereas pathogen receptor agonists (imiquimod) are approved to treat skin disorders [16]. Phosphodiesterase-4 inhibitors such as apremilast, approved for treatment of psoriatic arthritis, demonstrate the utility of modulating intracellular targets within cytokine signaling networks. Given the central role of kinases in cellular networks that control cytokine production and signaling, it is likely that novel kinase inhibitors will be important for treating autoimmune/auto-inflammatory disorders going forward [17].

Although inhibitors of protein kinases have been developed largely for neoplastic disorders in recent years, the first drug of this class (rapamycin) initially obtained FDA approval for use as an immunosuppressant following organ transplantation. Rapamycin forms a ternary complex with FKBP12 and mTOR resulting in an immune cell state reminiscent of nutrient

starvation [14]. A consequence is suppression of T and B cell responses normally elicited by activation of antigen receptor and/or IL-2 signaling. This seminal example illustrates the ability of kinase modulators to disrupt coordinately multiple signals needed for lymphocyte activation. The more recent approval of the JAK3 inhibitor tofacatinib for treatment of RA illustrates how small molecules can target redundancies within cytokine signaling networks. JAK3 preferentially associates with the common gamma chain (γ_c), which is a shared component of the receptor for IL-2 and many other cytokines (Figure 1b) [18]. Blocking γ_c/JAK3 signaling with tofacatinib affects several immune processes including reducing survival of activated T cells [19].

In addition to suppressing inflammatory cytokine function, kinase inhibitors may be exploited to stimulate production of anti-inflammatory cytokines such as IL-10. The importance of the IL-10 pathway in IBD is evidenced by disease-associated polymorphisms near *IL10* and its receptor (*IL10RA*), as well as near genes that control its production, such as *PTGER4* (which encodes the EP4 prostanoid receptor) and the transcriptional co-activator *CRTC3* [20]. Salt-inducible kinase 2 (SIK2) normally suppresses IL-10 production by phosphorylation of CRTC3 (CREB-regulated transcriptional co-activator 3), which results in its cytosolic sequestration by 14-3-3 proteins (Figure 1c) [21,22]. Stimuli that enhance cAMP levels (e.g., prostaglandin E2 or PDE4 inhibitors) suppress SIK2 activity and robustly potentiate IL-10 production by macrophages and dendritic cells (DCs), a phenotype that can be mimicked by small molecules that directly inhibit SIK2 [21,22]. Whereas recombinant IL-10 supplementation is ineffective in Crohn's disease (CD) patients [23], perhaps due to insufficient delivery to the gut mucosa [24], these data suggest that SIK2 inhibition may be effective at increasing IL-10 levels directly in this tissue. The additional ability of SIK2 inhibitors to suppress production of IL-12 and other inflammatory cytokines makes this kinase a promising target for further investigation in IBD.

Studies from genetics, physiology and chemical biology continue to implicate kinases as potential targets for restoring normal cytokine function in disease (Table 1). Novel polymorphisms in leucine-rich repeat kinase 2 (*LRRK2*, a gene previously linked to Parkinson's disease) confer increased risk of IBD [25]. Functional studies suggest that LRRK2 regulates production of reactive oxygen species and inflammatory cytokines by macrophages [26,27]. In addition, SNPs near *IRAK1*, a kinase required for production of interferons (IFNs) following viral infection, confer increased risk of systemic lupus erythematosus [28]. The serum/glucocorticoid-regulated kinase 1 (SGK1) regulates differentiation of T_H17 cells, a $CD4^+$ T cell subset that produces IL-17A and other inflammatory cytokines, in response to environmental factors including NaCl; small-molecule inhibition of SGK1 suppresses high salt-induced T_H17 development [29,30]. Mechanism-of-action studies have implicated the phoshatidylinositol kinase PIKfyve as the target of the clinical candidate apilimod, an inhibitor of IL-12/23 production discovered through phenotypic screening [31,32]. Targeting kinases implicated in cytokine regulation, with novel inhibitors or those repurposed from other indications, is a critical step for testing novel therapeutic hypotheses and may yield valuable starting points for drug development.

Controlling inflammation by targeted modulation of transcription

Signaling cascades downstream of immune receptors converge on transcription factors to regulate cytokine expression. The clinical success of calcineurin inhibitors, which suppress IL-2 production following T cell receptor stimulation by preventing dephosphorylation of NFAT [15], demonstrates the effectiveness of small molecules that target transcriptional regulation in immune cells. In addition to acute transcriptional responses, activation of immune cells leads to chromatin modifications that can promote acquisition of distinct effector states [6-8]. Genomic studies correlating transcription factor binding and histone modifications with gene expression have identified super-enhancers and other chromatin features that regulate immune cell function [33-35]. These insights, coupled with new tools for targeting transcription factors and chromatin-modifying proteins (Table 1), suggest that small-molecule modulators of transcription will be useful for therapeutic manipulation of cytokine networks.

RORγt (retinoid-related orphan receptor γt) is a nuclear hormone receptor (NHR) implicated in CD by human genetics that promotes differentiation of T_H17 cells (Figure 1d) [20,36]. Although a monoclonal antibody targeting IL-17A (secukinumab) has demonstrated potential for treating psoriasis and ankylosing spondylitis, it is ineffective in CD patients [37]. The failure of IL-17A blockade in CD may suggest the need to suppress a wider set of cytokines produced by T_H17 cells, possibly by interfering with T_H17 differentiation. RORγt contains a deep binding pocket for endogenous small-molecule ligands, which has facilitated development of RORγt antagonists that suppress T_H17 cell differentiation and display efficacy in murine models of graft-versus-host disease, demyelinating neurological disorders and cutaneous inflammation [38,39].

Their established roles in immune cell function, coupled with their ability to bind small molecules, make other NHRs intriguing drug targets. Activation of the retinoic acid receptor (RAR) by vitamin A metabolites enhances development of anti-inflammatory $CD4^+$ regulatory T cells (T_{reg}'s), an effect that contribute to the therapeutic activity of all-trans retinoic acid in murine models of autoimmune disease [40]. Binding of the aryl hydrocarbon receptor (AhR) by the tryptophan metabolite kynurenine stimulates IL-10 production by DCs and promotes differentiation of T_{reg}'s [41,42]; two mechanisms that may underlie the finding that sub-lethal doses of bacteria enhance resistance to subsequent infections [43].

NHRs often work in concert with chromatin-modifying enzymes, several classes of which have been targeted with small molecules to modulate cytokine production. The novel small-molecule inhibitor of the jumonji family histone demethylases JMJD3 and UTX (GSK-J4) suppresses inflammatory cytokine production in macrophages [44]. Pan-histone deacetylase (HDAC) inhibitors suppress inflammatory cytokine production by macrophages, promote T_{reg} differentiation and display efficacy in murine models of inflammation [45]. Of note, physiological concentrations of the microbial metabolite and pan-HDAC inhibitor butyrate specifically suppress IL-6, IL-12 and nitric oxide production in gut macrophages suggesting that HDAC inhibition may serve to limit auto-inflammatory responses to commensal microbes [11]. While *Hdac3*$^{-/-}$ macrophages display reduced inflammatory cytokine production [46], selective deletion of HDAC3 in intestinal epithelial cells alters intestinal

architecture and increases sensitivity to experimentally induced colitis [47]. Defining the tissue- and cell-specific functions of individual HDACs, coupled with development of isoform-selective HDAC inhibitors [48], will be needed to discover optimal therapeutic strategies for targeting this class of chromatin modulators.

Proteins that recognize differentially modified histones and transcription factors to effect changes in cell state may themselves be promising points of intervention. For example, the BET (bromodomain and extra terminal) family member BRD4 associates with the master regulator of inflammatory cytokine production NF-κB following acetylation at Lys310 [49]. Disrupting this interaction with the small-molecule pan-BET inhibitor I-BET762 suppresses inflammatory cytokine production by macrophages and protects mice from bacteria-induced sepsis [50]. In addition, inhibiting BRD4 with I-BET762 or (+)-JQ1 is protective in murine models of demyelinating disease by suppressing development of T_H1 and T_H17 cells [51,52], which are responsible for production of IFNγ and IL-17A, respectively..

Future perspectives: emerging targets from other protein classes

The success of biopharmaceuticals has validated modulation of cytokine function as a therapeutic approach in autoimmune/auto-inflammatory disorders. However, there are clear examples (e.g., IL-10 supplementation; IL-17A blockade in CD) where manipulation of individual cytokines has been ineffective, and studies of the genetics and physiology of these disorders has identified many intracellular proteins that contribute to disease pathogenesis. A desire to overcome these challenges has renewed interest in using in the historically productive approach of regulating cytokine networks with small molecules.

To date, small-molecule regulation of cytokine function has primarily focused on established targets like kinases and transcriptional regulators. However, recent studies are pointing to other protein classes as targets for treating autoimmune/auto-inflammatory disorders. Components of the ubiquitin-proteasome system (e.g., *TNFAIP3*) are critical for cytokine and pathogen receptor signaling, and have been linked to IBD, SLE, RA and type 1 diabetes by genetics [17]. In addition, the discovery of risk and protective alleles for IBD in *CARD9* suggests that scaffolding proteins may likewise be useful points of intervention [53]. Although traditional drug discovery has little experience with many emerging classes of targets, recent innovations in small-molecule science suggest that significant advances in this field will be forthcoming.

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Box 1

Using human disease genetics to guide drug development

The study of human genetics can uncover factors that contribute to the initiation and maintenance of disease, and suggest new strategies for therapeutic intervention. Therapies directed at correcting defects associated with causative alleles in Mendelian disease – protein therapeutics targeting IL-1β in cyropyrin disoders (see main text) or ivacaftor to modulate the *CFTR*-G551D allele in cystic fibrosis – illustrate the potential of genetics to inform discovery of effective medicines. In contrast to Mendelian diseases, many autoimmune/auto-inflammatory diseases have a complex genetic architecture in which susceptibility is influenced by multiple alleles as well as environmental factors. For instance, a recent genome-wide association study of inflammatory bowel disease (IBD) identified single nucleotide polymorphisms (SNPs) in 163 genetic loci (i.e., chromosomal regions) associated with altered disease risk [20]. Leveraging these insights for drug discovery will require understanding how disease genes contribute to pathophysiology. For example, the ATG16L-T300A SNP that confers increased risk of Crohn's disease (CD) is associated with defects in bacteria clearance and aberrant inflammatory cytokine production [54,55]. Small molecules that correct these defects may be useful for treating CD. While potentially less straightforward than monogenic diseases, the fact that several FDA-approved drugs have been shown retrospectively to modulate genes with risk-associated polymorphisms (e.g. thiazolidinediones targeting PPARγ for treatment of type 2 diabetes) and the early evidence of success for emerging targeting (e.g., PCSK9 in cardiovascular disease) suggests the approach may extend to complex inherited diseases (reviewed in [56]).

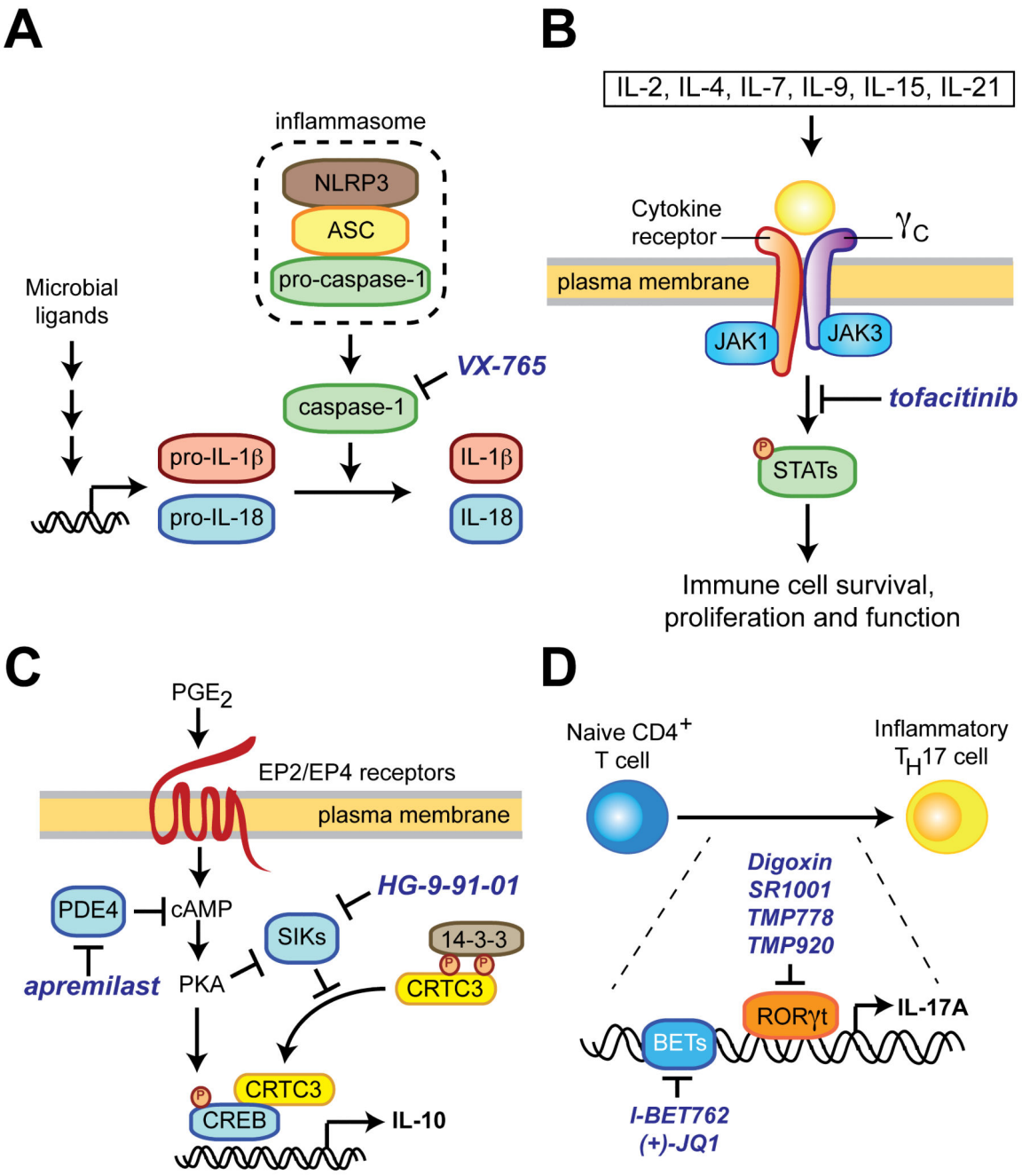

Figure 1.

Representative pathways regulating cytokine production and signaling that have been targeted by small molecules. (A) Microbial stimulation of innate immune cells induces transcription of full-length IL-1β and IL-18. Additional stress signals trigger inflammasome assembly leading to proteolytic processing of IL-1β and IL-18 to their mature forms by caspase-1. VX-765 and other caspase-1 inhibitors specifically block the final stage in this process. NLRP3, NOD-like receptor family, pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD. (B) The common

gamma chain (γ_c) is a shared component of the receptor for IL-2 and many cytokines and preferentially associates with Janus kinase-3 (JAK3). Stimulation of γ_c triggers JAK3-dependent phosphorylation of STAT family transcription factors, which leads to pleiotropic effects on immune cell function. Suppression of γ_c signaling with the small-molecule JAK3 inhibitor tofacitinib is approved therapy for rheumatoid arthritis. STAT, signal transducer and activator of transcription. (C) Salt-inducible kinases (SIKs) restrain IL-10 production by phosphorylation of CRTC3 (CREB-regulated transcriptional co-activator 3), which results in its cytosolic sequestration by 14-3-3 proteins. Stimuli that elevate intracellular cAMP levels (e.g., prostaglandin E2 or PDE4 inhibitors) suppress SIK activity and robustly potentiate IL-10 production by macrophages and dendritic cells, a phenotype that can be mimicked by small molecules that directly inhibit SIKs such as HG-9-91-01. PKA, protein kinase A. (D) Differentiation of inflammatory T_H17 cells requires the activity of the transcription factor RORγt and the chromatin-binding BET proteins.

Table 1

Examples of small molecule modulators of cytokine function

Target protein	Small molecule	Effects of target modulation on immune cell function	Approval status
Kinases and phosphatases			
mTOR	rapamycin	Inhibits T and B cell activation; promotes T_{reg} differentiation [14]	Approved for suppression of transplant rejection
Calcineurin	Cyclosporine A; FK506	Inhibits NFAT-mediated IL-2 production [15]	Approved for suppression of transplant rejection
JAK3	Tofacitinib [19]	Inhibits cytokine via receptors using the common gamma chain (γ_c) [18]	Approved for RA
SGK1	GSK650394 [57]	Inhibits IL-17A production and other phenotypes in salt-induced T_H17 cells [29,30].	Pre-clinical
Lyp/PTPN22	LTV-1 [58]	Potentiates T and B cell responses [58,59]	Pre-clinical
PIKfyve	Apilimod [32]	Inhibits TLR-induced IL-12p40 production [31,32]	Phase II for Crohn's Disease; Phase I for Psoriasis
SIK2	HG-9-91-01 [21]	Potentiates IL-10 production [21,22]	Pre-clinical
IRAK1	IRAK1/4inh [60]	Inhibits virus-stimulated interferon production [61]	Pre-clinical
LRRK2	GNE-7915 and others [62]	Reducing LRRK2 levels suppresses inflammatory cytokine production [26,27]	Pre-clinical
Chromatin modulators			
HDACs	TSA, SAHA and others [63]	Inhibits inflammatory cytokine production; increase T_{reg} survival/function [64]	Pre-clinical
JMJD3 and UTX	GSK-J4 [44]	Inhibits inflammatory cytokine production [44]	Pre-clinical
BET proteins	(+)-JQ1, I-BET762 and others [49]	Inhibits inflammatory cytokine production [65] [50] [51] [52]	Pre-clinical; phase II for type 2 diabetes (I-BET762)
RORγt	Digoxin, SR1001 and others [38,39]	Inhibits T_H17 cells differentiation [38,39]	Pre-clinical
AhR	TCDD, kynurenines and others [41,42]	Stimulates IL-10 production; promotes T_{reg} differentiation [41,42]	Pre-clinical
RAR	ATRA and others	Promotes T_{reg} differentiation; inhibits T_H17 cell differentiation [40]	Pre-clinical
Proteases			
Caspase-1	VX-765 [66]	Inhibits proteolytic processing of IL-1β and iL-18 to secreted forms [67]	Phase I for Muckle-Wells syndrome; Phase II for psoriasis

Target protein	Small molecule	Effects of target modulation on immune cell function	Approval status
Immuno-proteasome	ONX 0914 and others [68]	Inhibits antigen processing and inflammatory cytokine production (IL-23; IFNγ; IL-2) [69].	Pre-clinical
Regulators of second messengers			
PDE4	Apremilast and others [24]	Inhibits cAMP degradation [70]	Approved for psoriatic arthritis
Receptors			
Toll-like receptors	Rintatolimod (TLR3) [71]; imiquimod (TLR7) [72]; IMO-2125 (TLR9) [73]	Stimulates inflammatory cytokine production (e.g. type I IFNs) [16].	Phase II for HIV infection (Rintatolimod); Approved for treatment of skin disorders (imiquimod); phase I for Hepatitis C (IMO-2125)
IL-2/IL-2R	SP-4206 and others [74]	Inhibit IL-2 binding to IL-2R [75]	Pre-clinical
Prostanoid receptors	EP4 selective agonists [10]	Stimulates intracellular cAMP levels [22]	Phase II for ulcerative colitis