A Multitude of Kinases—Which are the Best Targets in Treating Rheumatoid Arthritis?

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The advent of biologic therapeutics, most notably anti–tumor necrosis factor (TNF) agents, has dramatically improved the treatment of rheumatoid arthritis (RA). Nevertheless, the available biologics rarely result in disease remission and provide clinical benefit only in subsets of RA patients. In addition, biologics can be administered only by injection and are expensive. Alternative therapies for RA are needed, and small-molecule kinase inhibitors may fit the bill. Small molecules have several features that give them the edge over other therapeutics: they are orally bioavailable, cell permeable, and inexpensive to manufacture. Insight into intracellular signaling pathways involved in inflammation and immunity has allowed the rational design of small molecules that can counteract aberrant immune responses. Small molecules can exert potent anti-inflammatory effects by inhibiting kinases, many of which lie at the nexus of multiple proinflammatory pathways. The therapeutic potential of kinase inhibitors is showcased by their success in the treatment of cancer.

Adaptive and innate immune responses are involved in the pathogenesis of RA, a systemic autoimmune disease characterized by destruction of the synovial joints. Initiation of the disease involves systemic dysregulation of T- and B-cell responses, which leads to a breach in self-tolerance and eventually to the mounting of an immune response against the synovial joints. During the chronic inflammatory stage of the

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disease, mast cells, macrophages, neutrophils, T cells, and B cells all infiltrate the synovium, where they release proinflammatory cytokines and matrix metalloproteinases (MMPs) that erode the synovial cartilage. Inflammation in the joints also triggers the development of apoptosis-resistant, hyperproliferative, fibroblast-like synoviocytes (FLS), which produce further proinflammatory cytokines. The synovial hyperplasia, in turn, leads to the formation of a destructive pannus that invades surrounding cartilage and bone. Finally, inflammation suppresses the formation of bone-forming osteoblasts and augments the formation of bone-resorbing osteoclasts, leading to the erosion of bone. Several kinases have been shown to play important roles in one or more of these pathogenic processes.This article discusses the therapeutic potential of small molecules targeting specific protein kinases in the treatment of RA and provides an overview of the progress to date. Lipid kinases—in particular, the phosphoinositide-3 kinases (PI3Ks)—are also emerging as attractive drug targets in the treatment of inflammation. The therapeutic potential of blocking PI3Ks in RA has been recently reviewed[1] and is not discussed further.

MITOGEN-ACTIVATED PROTEIN KINASES: ADVANCES AND SETBACKS

Mitogen-activated protein kinase (MAPK) signaling comprises 3 interrelated pathways, each mediated by a distinct MAPK: p38, extracellular signal-regulated kinase (ERK), or c-Jun N-terminal kinase (JNK). These pathways involves the sequential activation of multiple kinases, such that the MAPKs are activated by MAPK kinases (MKKs), which are themselves activated by MAPKK kinases (MKKKs). Thus, the p38 kinases (α, β, γ, and δ) are activated by MKK3 and MKK6, the ERKs (1 and 2) by MAPK-ERK kinase (MEK) 1 and MEK2, and the JNKs (1, 2, and 3) by MKK4 and MKK7.[2] JNK, ERK, and p38 are the terminal kinases of these pathways and serve to regulate an array of cellular responses through the phosphorylation of serine/threonine residues in discrete sets of transcription factors. All 3 of these MAPKs are activated in RA synovium[3] and have been proposed as therapeutic targets in the treatment of RA.

P38

Enthusiasm for inhibitors of p38—until recently heralded as one of the most promising classes of oral therapeutics for RA—has finally subsided. Many p38 inhibitors have been developed and tested in preclinical and clinical studies. Although the preclinical data were encouraging, with p38 inhibition shown to suppress inflammation and joint destruction in many different models of RA,[4] these initial successes did not extend to the treatment of RA. The first generation of small-molecule p38 inhibitors, which targeted all 4 isoforms of p38, failed in clinical trials owing to liver, brain, and skin toxicities. Nevertheless, the discovery that p38α is the important isoform in RA, acting to drive the expression of proinflammatory cytokines and the formation of osteoclasts,[5,6] engendered hope that selective inhibition of p38α would avoid the adverse effects of the pan-38 inhibitors. Unfortunately, p38α-specific inhibitors did not perform much better (**Table 1**). For instance, clinical development of SCIO-323 and AMG-548 was terminated because of skin toxicity and liver toxicity, respectively,[7] and the p38α inhibitors that did advance to phase II clinical trials proved ineffective.[8,9]

The toxicity and the inefficacy of p38 inhibitors are most likely target based, rendering the systemic targeting of p38 unviable. Multiple structurally unrelated p38 inhibitors have been shown to be toxic to the liver and skin and to induce only transient reductions in markers of inflammation.[4,7] The pivotal position of p38α in the regulation of inflammation is thought to underlie these phenomena. Although its proinflammatory

Table 1
Small-molecule kinase inhibitors in RA clinical trials

Kinase Target	Inhibitor (Company)	Outcome in RA Clinical Trials
p38α	SCIO-323 (Scios)	Clinical development terminated owing to skin toxicity[7]
	AMG-548 (Amgen)	Clinical development terminated owing to liver toxicity[7]
	Pamapimod (Roche)	Phase II trial comparing efficacy and safety with MTX treatment[8] • ACR20 response rate was higher in the MTX-treated group than in the pamapimod-treated group
	VX-702 (Vertex)	Phase II trial in patients with RA on a background of MTX[9] • Tolerated but no significant increase over placebo in ACR20 response rate
JAKs	INCB18424 (Incyte)	Phase II trial in patients with RA on a background of MTX, SSZ, antimalarials, or prednisone[a] • ACR20, ACR50, ACR70, and ACR90 response rates were 83%, 50%, 25%, and 17%, respectively, in the 15-mg group, compared with 33%, 11%, 0%, and 0% in the placebo group
	CP690550 (Pfizer)	Phase II trial in patients with RA refractory to MTX, etanercept, infliximab, or adalimumab[59] • ACR20 response rates were 70.5%, 81.2%, and 76.8% in the 5-mg, 15-mg, and 30-mg groups, respectively, compared with 29.2% in the placebo group • ACR50 and ACR70 response rates were significantly greater in all treatment groups compared with the placebo group
Syk	R788 (Rigel)	Phase II trial in patients with RA on a background of MTX, SSZ, antimalarials, prednisone, or NSAIDs[64] • ACR20, ACR50, and ACR70 response rates were 72%, 57%, and 40%, respectively, in the 150-mg group compared with 38%, 19%, and 4% in the placebo group
MEK 1/2	ARRY-162 (Array BioPharma)	Phase II trial in patients with RA refractory to MTX[b] • Tolerated but no significant increase over placebo in ACR20 response
c-Kit/PDGFR	AB1010 (AB Science)	Open-label, uncontrolled, phase II trial in patients with RA refractory to MTX or anti-TNF agents[85] • ACR20, ACR50, and ACR70 response rates were 54%, 26%, and 8%, respectively • High rate (37%) of patient withdrawal owing to adverse effects
	Imatinib mesylate (Novartis)	Phase II trial in patients with active RA on a background of MTX[c] • Outcomes not reported

Abbreviations: MTX, methotrexate; SSZ, sulfasalazine.

[a] Incyte press release. Incyte's JAK inhibitor demonstrates marked clinical benefits in phase IIa rheumatoid arthritis study. Available at: http://www.incyte.com/Eular%20PR%206%2012%2008%13-%20Final.pdf. Accessed January 25, 2010.

[b] Array BioPharma. Array Biopharma announces top-line results from rheumatoid arthritis phase 2 trial. Available at: http://www.drugs.com/clinical_trials/array-biopharma-announces-top-line-results-rheumatoid-arthritis-phase-10-trial-7991.html. Accessed January 25, 2010.

[c] Novartis Pharmaceuticals. A study of imatinib 400 mg once daily in combination with methotrexate in the treatment of rheumatoid arthritis. Available at: http://www.clinicaltrials.gov/ct2/show/NCT00154336?term=imatinib+and+rheumatoid&rank=. Accessed January 25, 2010.

role has long been recognized, p38α has more recently been found to play an anti-inflammatory role also. Not only does it drive the expression of important anti-inflammatory genes but also it mediates intracellular feedback loops that constrain the activity of other proinflammatory pathways. For instance, p38α activates mitogen- and stress-activated protein kinase (MSK) 1 and MSK2, which contribute to the resolution of inflammation through the transcriptional activation of anti-inflammatory genes, such as interleukin (IL)-10, IL-1 receptor antagonist, and protein phosphatase dual specificity.[10–12] p38α also reigns in inflammation by phosphorylating TGF-beta-activated kinase (TAK) -associated kinase 1 (TAB1), thereby inhibiting TAK1, which regulates the proinflammatory JNK and inhibitor of κB (IκB) kinase (IKK) pathways, as well as p38α itself.[4] Thus, blockade of p38α would allow inflammation to proceed unchecked. Genetic evidence supports the idea that p38α inhibition underlies the toxicity and inefficacy of p38 inhibitors: myeloid cell-specific ablation of p38α in mice results in increased ERK and JNK activity and in vascular permeability and edema[12]; double deficiency in MSK1 and MSK2 leads to prolonged inflammation in a model of toxic contact eczema[10]; and hepatocyte-specific ablation of p38α in mice results in excessive activation of the proapoptotic JNK in the liver after lipopolysaccharide (LPS) challenge.[13]

Although the death knell may have sounded for inhibitors of p38, components downstream of p38α may yet constitute viable therapeutic targets. MAPK-activated protein kinase 2 (MK2), a kinase downstream of p38α that post-transcriptionally promotes the expression of proinflammatory genes, has been proposed as one such candidate.[4] Targeting of MK2 should spare p38α-mediated anti-inflammatory mechanisms, including the p38α-TAB1 feedback loop and expression of anti-inflammatory genes. Support for such an approach comes from the finding that MK2-deficient mice are protected against collagen-induced arthritis (CIA).[14] One small-molecule MK2 inhibitor has already been shown to reduce LPS-induced TNF production in rats, and many more are being synthesized.[15,16]

MEKs and ERKs

The critical role of MEK-ERK signaling in cell proliferation has led to MEK1 and 2 and ERK1 and 2 being investigated as candidate targets in clinical trials in cancer.[2] MEK-ERK signaling is upregulated in synovial tissues in RA and in CIA[17] and promotes proliferation of RA FLS in vitro.[18] MEK-ERK signaling may thus contribute to the pathogenesis of RA by driving formation of the tumor-like pannus that is characteristic of RA. But the MEK-ERK cascade is not solely a proliferative one—it is also proinflammatory, inducing the production of IL-1β, IL-6, TNF, and MMPs, and itself is activated by proinflammatory cytokines. In addition to promoting inflammation and tissue destruction in the synovial joints, ERK signaling is important in lymphocyte activation and differentiation. ERK mediates B-cell receptor (BCR) and CD40 receptor signaling in B cells and T-cell receptor signaling in T cells.[19,20] Recent data suggest that ERK dysregulation in $CD4^+$ and $CD8^+$ T cells may even contribute to the breakdown of T-cell tolerance in RA by lowering the threshold for T-cell activation.[19]

Several small-molecule inhibitors of MEK 1 and 2 have shown efficacy in animal models of RA (**Table 2**). Oral administration of PD184352 to mice with CIA suppressed synovitis, pannus formation, and cartilage and bone erosion.[17] These effects correlated closely with the inhibition of ERK phosphorylation in mouse joints. PD184352 also prevented proteoglycan loss in articular cartilage in a rabbit model of IL-1b-induced arthritis.[17] Prophylactic, intraperitoneal administration of subtherapeutic doses of U0126 to SKG mice, which spontaneously develop autoimmune arthritis owing to a mutation in *zap70*,[21] delayed disease onset and reduced disease severity,

Table 2
Small-molecule kinase inhibitors showing treatment benefit in animal models of RA

Kinase Targeted	Small-Molecule Inhibitor (Company)	RA Model
MEK 1/2	PD184352 (Pfizer)	Mouse CIA; rabbit cytokine-induced cartilage degradation[17]
	U0126 (DuPont Pharmaceuticals)	Mouse SKG[19]
	ARRY-162[a] (Array BioPharma)	Rat CIA and AIA[22]
ERK 1/2	FR180204 (Astellas)	Mouse CIA[23]
JNK 1/2/3	SP600125 (Celgene)	Rat AIA[40]
	AS601245 (EMD Serono)	Mouse CIA[42]
IKK2	BMS-345541 (Bristol-Myers Squibb)	Mouse CIA[95]
	BMS-066 (Bristol-Myers Squibb)	Mouse CIA and rat AIA[96]
	TPCA-1 (GlaxoSmithKline)	Mouse CIA[97]
	PHA-408 (Pfizer)	Rat SCWA[98]
	ML120B (Millenium Pharmaceuticals)	Rat AIA[99]
	IMD-0560 (IMDD)	Mouse CIA[100]
	SPC839 (Celgene)	Rat AIA[101]
c-Fms	GW2580 (GlaxoSmithKline)	Rat AIA[78]; mouse CIA, CAIA, K/BxN[74]
	Ki20227 (Kirin)	Mouse CIA[79]
	Cyanopyrrole 8 (Johnson & Johnson)	Mouse CIA[80]
BTK	Compound 4 (Celera Genomics)	Mouse LPS-induced arthritis[87]
	Cgi1746 (CGI Pharmaceuticals)	Mouse CIA[86]
PI3Kγ	AS-604850, AS-605240 (EMD Serono)	Mouse CAIA[104]

Abbreviations: IMDD, Institute of Medicinal and Molecular Design; PI3Kγ, phosphoinositide-3 kinase γ; SCWA, streptococcal cell wall–induced arthritis.
[a] In clinical trials in RA patients (see **Table 1**).

supporting the concept that ERK dysregulation may contribute to the development of RA.[19] A third MEK1 and 2 inhibitor, ARRY-162, inhibited inflammation and bone resorption in mice with CIA and in rats with adjuvant-induced arthritis (AIA) and exhibited additive efficacy when combined with standard-of-care agents such as anti-TNFs and methotrexate.[22] These promising findings saw ARRY-162 enter clinical development; however, despite being well tolerated, ARRY-162 did not fare any better than placebo in a recent phase II, 12-week trial in patients with active RA on a background of methotrexate treatment (see **Table 1**).

In addition to inhibitors of MEK1 and 2, an inhibitor of the downstream ERKs has been assessed in a mouse model of RA. Intraperitoneal administration of the ERK 1 and 2 inhibitor FR180204 to mice before the induction of CIA reduced the clinical signs of arthritis, the production of anti-collagen type II (CII) antibodies, and CII-specific proliferation of T cells.[23] Conversely, recent studies suggest that targeting components upstream of MEK may also provide efficacy in RA. Tumor progression locus 2 (TPL2) is the MKKK that activates MEK1 and 2 and hence the ERKs.[24,25] Studies using *tpl2*$^{-/-}$ mice have shown that TPL2 is required for LPS-induced production of circulating TNF in vivo and for LPS-induced production of TNF by macrophages in vitro.[24,26] Furthermore, TPL2 deficiency has been shown to protect mice from TNF-induced inflammatory bowel disease[27] and arthritis (unpublished data with G. Kollias cited in Das and colleagues[28]). Several small-molecule inhibitors of TPL have been assessed for their ability to suppress TPL2-MEK-ERK–induced inflammation. Compound 1 suppressed LPS- and IL-1β–induced production of TNF by human monocytes as well as IL-1β–induced production of IL-6, IL-8, prostaglandin E2, and MMPs by RA FLS.[29] Compound 44 inhibited the production of TNF in an LPS-induced

model of inflammation in rats.[30] Results from the testing of TPL2 inhibitors in animal models of RA have not been described to date.

Thus, small-molecule inhibitors exist for the targeting of the TPL2-MEK-ERK pathway at 3 different levels. The inefficacy of the MEK1 and 2 inhibitor ARRY-162 in a phase II RA trial, however, together with concerns that MEK/ERK inhibition could result in the development of lupus-like disease,[31–33] raise doubts over the potential of MEK/ERK inhibitors for the treatment of RA. Safety might also be an issue with TPL2 inhibitors, but these could potentially provide greater therapeutic efficacy than MEK/ERK inhibitors. Although the signaling defect in TPL2-deficient macrophages and B cells seems restricted to activation of the MEK-ERK pathway,[24,25] TPL2 regulates the activation of JNK and nuclear factor κB (NF-κB), in addition to ERK, in mouse embryonic fibroblasts.[28] Because synovial-fibroblast production of proinflammatory and degradative mediators is important in the pathogenesis of RA, inhibition of TPL2 might provide added benefit by suppressing ERK-driven activation of lymphocytes and ERK-, JNK-, and NF-κB–driven activation of synovial fibroblasts.

JNKS

Activated by stress signals and cytokines, JNKs play important roles in apoptosis, inflammation, and matrix degradation.[34,35] JNKs exist as 3 isoforms: JNK1, JNK2, and JNK3. JNK1 and JNK2 are ubiquitously expressed, and phosphorylation of these isoforms is detected in RA synovium but not in osteoarthritic synovium[36]; JNK3 expression is largely restricted to the brain, heart, and testes, and, therefore, not thought to be involved in RA.[37,38] As discussed later, some of the efficacy of spleen tyrosine kinase (Syk) inhibitors in RA could potentially be attributed to the inhibition of JNKs, because the tyrosine kinase Syk lies upstream of JNK in the MAPK signaling cascade. Syk-activated JNKs drive the expression of IL-6 and MMP-3 in RA FLS.[39] Induction of MMP expression is defective in JNK1- or JNK2-deficient murine FLS, and pharmacologic inhibition of JNK blocks induction of MMP expression in RA FLS.[40] In addition to promoting synoviocyte production of proinflammatory mediators, JNK1 regulates the differentiation of T cells into Th1 cells.[41]

The JNK-driven expression of MMPs seems critical in the destruction of joints in inflammatory arthritis. Subcutaneous administration of SP600125, a small-molecule inhibitor that targets all 3 JNK isoforms, suppressed cartilage and bone erosion in rat AIA, effects associated with inhibition of JNK activity and MMP expression in the joints.[40] Oral administration of another pan-JNK inhibitor, AS601245, attenuated CIA in mice, reducing synovial inflammation and cartilage degradation.[42] JNK1 deficiency, however, does not confer resistance to destructive arthritis in JNK1-deficient, TNF-transgenic mice nor does it reduce the activity of JNK-mediated signaling.[43] In addition, JNK2 deficiency confers only modest protection against the development of anti–collagen antibody-induced arthritis (CAIA).[40] Together, these findings suggest that inhibition of both JNK1 and JNK2 is required for the effective attenuation of inflammatory arthritis.

Although developed as a JNK inhibitor, SP600125 has been shown to inhibit 13 other protein kinases with similar or greater potency and to have an unfavorable pharmacokinetic profile.[36,44] Likewise, AS601245 exhibits only moderate selectivity for JNK.[42] More specific inhibition of the JNK signaling cascade can be achieved by targeting the physical interaction between JNK and other components of the cascade. JNK-interacting protein 1 (JIP1) is a scaffolding protein that promotes JNK activity by facilitating the interaction between JNK and upstream kinases.[45] Overexpression of JIP1, however, suppresses JNK activity (presumably owing to the sequestration of

JNK-interacting components), and a peptide corresponding to the minimal region of JIP1 (pepJIP1) has been developed as an inhibitor of JNK.[46] Although peptide therapeutics are associated with certain disadvantages, such as their rapid degradation in vivo and the need for administration via injection, a small-molecule mimic of pepJIP1, BI-78D3, was recently developed and shown to exert anti-inflammatory effects in vivo, restoring insulin sensitivity in a mouse model of type 2 diabetes.[47] In addition, a small-molecule inhibitor that selectively blocks the DNA-binding activity of AP-1, an important JNK-activated transcription-factor complex, was recently shown to be efficacious in a mouse model of arthritis. Oral administration of the AP-1 inhibitor T-5224 prevented and treated CIA in mice, abrogating joint destruction and suppressing MMP and IL-1β expression.[48]

Although toxicity in animal models treated with inhibitors of the JNK pathway has not been reported, long-term suppression of JNK could potentially have adverse effects due to JNK's role in regulating apoptosis.[35] JNK1-deficient mice spontaneously develop intestinal tumors and are more susceptible to the development of TPA-induced skin tumors.[49,50] Thus, increased tumorigenicity may limit the value of JNK inhibitors for the treatment of chronic inflammatory disorders, such as RA.

TYROSINE KINASES: THE FRONTRUNNERS

Tyrosine Kinases Targeted in RA Clinical Trials

Janus kinases

Janus kinases (Jaks) play important roles in innate and adaptive immune responses, serving to transduce signals from cytokine receptors that lack intrinsic kinase activity. Cytokine receptors containing the common γ-chain subunit signal through Jak1 and Jak3, whereas receptors for hematopoietic growth factors or gp40-containing cytokines signal through Jak2. Jak1 and Jak2 are ubiquitously expressed and are essential for lymphopoiesis and hematopoiesis, respectively.[51] Jak3 is expressed primarily in cells of the immune system and is critical in lymphocyte activation, function, and proliferation[52]; accordingly, the defect in Jak3-deficient mice seems to be restricted to T cells, B cells, and natural killer cells.[53,54]

Given their multifarious roles in innate and adaptive immunity, it might be expected that Jaks are involved in the pathogenesis of RA. It was not until recently, however, that Jaks began to be explored as candidate therapeutic targets in RA. Progress has since been rapid. The finding that inhibition of Jak3 ameliorates clinical signs of inflammatory arthritis by greater than 90% and protects against joint damage in rodent models of RA[55] was swiftly followed by assessment of the therapeutic efficacy of 2 small-molecule Jak inhibitors—CP690550 and INCB18424—in patients with RA. CP690550 was developed as a Jak3 inhibitor but also inhibits Jak2, albeit less potently; its selectivity for the Jaks has been confirmed by testing against a panel of 317 kinases.[56] INCB18424 is an inhibitor primarily of Jak1 and 2. High hopes are now pinned on these Jak inhibitors. They are arguably the best-performing investigational small-molecule drugs in RA at present, with CP690550 and INCB18424 proving efficacious and well tolerated in initial phase II clinical trials (CP690550, 6-week trial; INCB18424, 28-day trial) (see **Table 1**). Which of these 2 Jak inhibitors will prove to be safer in the long term remains to be seen. On the one hand, the restriction of Jak3 expression to hematopoietic cells might mean that a Jak3 inhibitor will have fewer target-based adverse effects than a Jak1 and 2 inhibitor; on the other hand, *Jak3* mutations in humans are known to cause severe immunodeficiency syndrome.[57,58] In addition, the nature of the adverse effects seen with CP690550 suggest that therapeutically efficacious doses of this compound result in inhibition of Jak2 in addition to Jak3.[59] Conversely, Jak3 signaling may be indirectly affected

by inhibitors of Jak1, because Jak1 and Jak3 cooperate in the transduction of multiple signals.[60] The outcomes of phase IIb trials of CP690550 and INCB18424 are eagerly awaited.

Syk

Another prime therapeutic contender is R788, the prodrug for the R406 small-molecule inhibitor of Syk. Syk is expressed in all hematopoietic cells, mediating immunoreceptor signaling, such as BCR signaling in B cells and FcγR signaling in mast cells, macrophages, neutrophils, and basophils.[61] It is also expressed in nonhematopoietic cells, in which it transduces signals from receptors for TNF, IL-1, and LPS. Syk activity is upregulated in RA synovium compared with control osteoarthritic synovium and mediates the production of IL-6 and MMP-3—major culprits in joint destruction—in TNF-stimulated RA FLS.[39] Syk also promotes osteoclast activity.[61] Thus, Syk may promote both the adaptive immune responses and the destructive effector processes that underlie RA, making it an attractive therapeutic target.

The R406 Syk inhibitor suppressed inflammation and joint destruction in 2 antibody-mediated models of RA in mice[62] as well as in a T-cell–mediated model of RA in rats.[63] In a preliminary 12-week phase II trial in RA, R788 (which is rapidly converted to R406 after oral administration) proved efficacious and generally well tolerated.[64] R788 administration resulted in a rapid and sustained decrease in serum IL-6 and MMP-3 levels, an indication that Syk inhibition may be able to halt joint damage. The long-term efficacy and safety of R788 is the focus of an ongoing open-label study of the RA patients who completed the initial R788 phase II trial. Although relatively specific for Syk,[62] R788 did cause hypertension in a few RA patients, which may reflect off-target inhibition of the vascular-endothelial growth factor receptor (VEGFR).[64] This observation has raised some concern about the safety of R788 in RA, a disease associated with increased cardiovascular complications.[65] As for target-mediated adverse effects, the ubiquity of Syk may be an issue, but its nonredundant functions in adulthood may not be as widespread as its expression.[61] Syk has been shown to signal upstream of JNK in mast cells[66] and in RA FLS[39]; therefore, Syk inhibition could potentially share some of the advantages and disadvantages of JNK inhibition (discussed previously).

Tyrosine Kinases Targeted in Animal Models of RA

Several other tyrosine kinases have been implicated in RA, partly on the basis of observations in cancer patients treated with imatinib mesylate (imatinib). Imatinib, the first kinase inhibitor introduced into clinical practice, targets several tyrosine kinases, including BCR-ABL, platelet-derived growth factor (PDGFR), c-Fms, c-Kit, Syk, and leukocyte-specific kinase. Case studies documented the alleviation of RA symptoms in patients administered imatinib for the treatment of chronic myelogenous leukemias or c-Kit–expressing gastrointestinal stromal tumors,[67,68] suggesting that one or more of the imatinib-targeted kinases are important in the pathogenesis of RA. Prompted by these findings, Eklund and Joensuu[69] administered imatinib to 3 patients with treatment-refractory RA. All 3 patients showed some degree of clinical improvement; 1 patient continued treatment for 24 months and showed marked and long-lasting clinical improvement.[70] Two of the 3 patients in this study, however, discontinued imatinib treatment at 2 and at 4 months, owing to adverse events. Furthermore, the outcomes of a double-blind, placebo-controlled, 3-month, phase II trial conducted by Novartis, in which imatinib was administered to patients with active RA despite methotrexate treatment, were never reported. Although toxicities—including cardiotoxicity due to inhibition of Abl[71]—may limit the use of imatinib in nononcologic chronic diseases, selectively inhibiting the imatinib-targeted kinases that are important in RA may provide

a more favorable risk-to-benefit ratio. In mouse studies, imatinib-induced attenuation of CIA was associated with suppression of c-Fms activation in synovial macrophages, of PDGFR activation in FLS, and of c-Kit activation in mast cells.[72] The involvement of each of these tyrosine kinases in RA has been independently investigated.

Accumulating evidence suggests that c-Fms and its ligand, macrophage colony-stimulating factor (M-CSF), are involved in the pathogenesis of RA. M-CSF–c-Fms signaling is integral to macrophage and osteoclast formation, as evidenced by the osteopetrosis and the reduction in tissue macrophages in M-CSF– and in c-Fms–deficient mice.[73] M-CSF levels are elevated in the synovial fluid and serum of RA patients,[74,75] and administration of exogenous M-CSF to mice exacerbates submaximal CIA.[76] Conversely, M-CSF–deficient mice are resistant to the development of CIA, and neutralizing antibodies against M-CSF or c-Fms attenuate mouse CIA.[76,77] Several small-molecule inhibitors of c-Fms have been developed and tested in models of RA. In parallel experiments, the c-Fms–specific inhibitor GW2580, was as efficacious as imatinib in attenuating inflammatory arthritis in antibody-mediated and T-cell–mediated mouse models of RA.[74] In these models, prophylactic, oral administration of GW2580 reduced synovitis, pannus formation, and cartilage and bone erosion; GW2580 was also able to treat established arthritis. The amelioration of arthritis was associated with reduced macrophage infiltration and c-Fms expression in the synovial joints. In vitro, GW2580 inhibited the differentiation of monocytes into macrophages and osteoclasts; the resorption of bone by osteoclasts; and the priming of TNF production in Fc receptor (FcR)-stimulated macrophages.[74] Thus, c-Fms inhibitors may have potential in the treatment of RA through the mitigation of the non–antigen-specific processes that underpin the chronic inflammatory stage of RA. GW2580 has also been shown to attenuate tissue and bone destruction in the joints of rats with AIA, although no effects on joint inflammation were detected in this model.[78] Two other orally bioavailable c-Fms inhibitors, Ki20027 and cyanopyrrole 8, have been shown to reduce joint inflammation and bone destruction in rodent models of RA, but these compounds are less selective than GW2580.[79,80] Tested against a panel of 179 kinases, GW2580 proved relatively selective, inhibiting only c-Fms (IC_{50} of 0.03 μM) and TrkA (IC_{50} of 0.88 μM).[78] The restriction of c-Fms expression to monocyte-lineage cells might mean that c-Fms inhibitors are relatively safe and well tolerated. Nevertheless, elevations in levels of liver enzymes in arthritic mice treated with GW2580, although not associated with histologic evidence of pathology, could indicate potential toxicities of GW2580.[78]

Although PDGFR and c-Kit have been implicated in RA, small-molecule inhibitors that selectively inhibit either one of these kinases are not currently available. PDGFR is a ubiquitous tyrosine kinase that plays a key role in fibroblast proliferation, and imatinib has been shown to inhibit PDGFR-mediated proliferation of FLS derived from arthritic mice or from RA patients.[72,81] Therefore, PDGFR is thought to contribute to RA pathogenesis by promoting synovial hyperplasia and, thus, pannus formation. c-Kit, has been proposed as contributing by mediating the aberrant activation of mast cells. c-Kit is essential for the survival and activation of mast cells, and release of proinflammatory mediators from synovial mast cells precedes the onset of clinical signs of inflammation in certain antibody-mediated models of RA.[82] The importance of mast cells in autoimmune arthritis is contentious, however. In initial studies, mouse strains deficient in mast cells—owing to a loss-of-function mutation in the gene encoding the c-Kit ligand ($Kit^{Sl}/Kit^{Sl\text{-}d}$ mice) or a mutation in *c-Kit* ($Kit^{W}Kit^{W\text{-}v}$ mice)—were shown to be resistant to arthritis induced by K/BxN serum transfer; moreover, engraftment of mast cells restored susceptibility to arthritis in these mice.[82] These findings cast mast cells as the cellular link between autoantibodies and arthritis.

Subsequent studies, however, showed that *Kit*$^{W\text{-}sh}$ mice, which are mast cell–deficient owing to a mutation that abrogates *c-Kit* expression specifically in mast cells, develop full-blown CAIA.[83] Thus, c-Kit may contribute to RA through effects in a cell type other than the mast cell. To date, the most potent and specific small-molecule inhibitor of c-Kit is masitinib (AB1010), with an IC_{50} of 200 nM for inhibition of recombinant c-Kit.[84] Masitinib, however, also inhibits PDGFR and LynB at nanomolar concentrations—although, unlike imatinib, it is a weak inhibitor of c-Fms and Abl. In a small, open-label, dose-ranging, 12-week, phase II trial in RA patients, masitinib exhibited only moderate efficacy (in the absence of a placebo control).[85] Furthermore, patient-withdrawal rate was high, owing to adverse effects. Thus, whether or not inhibiting c-Kit or PDGFR would be of therapeutic value in RA is currently unclear.

Another interesting kinase is Bruton's tyrosine kinase (BTK). It is expressed primarily in B cells, mast cells, platelets, and myeloid cells.[86] Mutations in the *BTK* gene result in X-linked agammaglobulinemia (XLA), a disease characterized by marked reduction in numbers of mature B cells and by severe immunodeficiency. BTK transduces BCR signaling in B cells, FcεR1 signaling in mast cells, and toll-like receptor (TLR) signaling in monocytes. Monocytes from XLA patients exhibit defective TNF production in response to TLR stimulation, and BTK-deficient mast cells exhibit impairment of degranulation, histamine release, and cytokine production.[86] A relatively selective BTK inhibitor, compound 4, was shown to be efficacious in an LPS-induced mouse model of RA, but its therapeutic use may be limited because it is an irreversible inhibitor.[86,87] Cgi1746, a reversible orally bioavailable BTK inhibitor with good selectivity, showed efficacy in mouse CIA.[86] In addition, the rationally designed BTK inhibitor LFM-A13—an analog of a metabolite of leflunomide (which is used to treat RA)—has been shown to suppress FcεRI-induced release of histamine from rat mast cells.[88] Encouragingly, preclinical studies have demonstrated favorable pharmacokinetic and toxicity profiles of LFM-A13 in mice, rats, and dogs.[89]

The tyrosine kinase VEGFR has also been implicated in RA and is reviewed elsewhere.[90] Therapeutic targeting of VEGFR may be associated, however, with cardiotoxicity and hypertension,[91] which may be of particular concern in RA, a disease that is often accompanied by cardiovascular dysfunction.

INHIBITOR OF κB KINASE 2: RESURGENCE OF AN OLD FAVORITE

The NF-κB pathway is considered the master regulator of inflammation and immunity. It plays a pivotal role in inflammatory and autoimmune diseases—no less so in RA. Several drugs used in the treatment of RA, including sulfasalazine, glucocorticoids, leflunomide, and gold compounds, can inhibit NF-κB. NF-κB is intimately involved in the autoimmune, inflammatory, and destructive processes that underlie RA.[92] It promotes (1) proliferation of T cells, by inducing the expression of IL-2; (2) antibody production and class switching in B cells; (3) recruitment of inflammatory cells, by inducing the expression of adhesion molecules and chemokines; (4) production of proinflammatory cytokines by multiple cell types; and (5) synovial hyperplasia, by driving angiogenesis and FLS proliferation and survival. In addition, NF-κB directly promotes erosion of cartilage and bone by 3 different mechanisms: it induces the expression of the matrix-degrading MMPs; it mediates the survival and differentiation of bone-resorbing osteoclasts; and it inhibits the formation of bone-forming osteoblasts. Underscoring the importance of NF-κB in inflammatory arthritis, mice deficient in the p50 or c-rel NF-κB subunits are resistant to the development of CIA,[93] as are transgenic mice overexpressing a super-repressor form of the NF-κB inhibitor IκBα in the T-cell lineage.[94]

The NF-κB transcription factor is regulated by the upstream IKK complex, consisting of the kinases IKK1 and IKK2 and the regulatory component NF-κB essential modulator (NEMO). IKK2 is the kinase that plays the dominant role in activation of the canonical, proinflammatory NF-κB pathway; thus selective inhibition of IKK2 has been explored as an anti-inflammatory therapeutic approach. Many orally bioavailable, small-molecule inhibitors of IKK2 have been shown to profoundly suppress the development and the progression of inflammatory arthritis in rodent models of RA.[95–101] Confirming that targeting of IKK2 underlies these effects, intra-articular gene transfer of a dominant-negative form of IKK2 was shown to attenuate rat AIA.[102] Although the importance of NF-κB in inflammation and immunity has long been recognized, IKK/NF-κB inhibitors have yet to make it into the clinic. The reason for this is that NF-κB is also important in normal physiology; chronically shutting down NF-κB is expected to incur several serious adverse effects, including increased susceptibility to infection and tissue injury due to generalized apoptosis. Nevertheless, recent findings suggest that IKK/NF-κB inhibitors may have better prospects than once thought. For instance, although NF-κB is indispensable for liver development in the fetus, it seems that inhibition of NF-κB in the developed liver is not hepatotoxic and may even be hepatoprotective.[92] Moreover, approaches allowing partial suppression of NF-κB activity are starting to yield promising results.

One such approach is the use of a cell-permeable peptide corresponding to the NEMO-binding domain (NBD) of IKK2 to disrupt the interaction of IKK2 with NEMO, thereby blocking the formation of the IKK complex. The NBD peptide has been shown to inhibit LPS-induced osteoclastogenesis in vitro and in vivo and to suppress inflammation and bone destruction in the joints of mice with CIA—without inducing any overt toxicity.[103] This favorable therapeutic index has been ascribed to the abrogation of inflammation-induced, but not basal, NF-κB activity. Consistent with this, the NBD peptide reduced serum and joint levels of TNF, IL-1β, and MMP-9 in CIA mice to those seen in naive mice. Because peptide therapeutics are beset by several disadvantages, the full potential of this approach may not be realized until compounds that mimic the effect of the NBD peptide are developed. Another approach to the partial inhibition of NF-κB involves simply administering submaximal doses of a small-molecule inhibitor of IKK2 activity. Periodic, oral, administration of the IKK2 inhibitor BMS-066 was recently shown to protect against the development of rat AIA and mouse CIA at doses that only partially and transiently inhibit NF-κB activity.[96] The observed protection was attributed to the cumulative effect of partial inhibition of multiple NF-κB–dependent pathogenic processes. Thus, the scope of NF-κB's role in immunity and inflammation, once thought to preclude the therapeutic targeting of the NF-κB pathway, may be turned to advantage. Dampening, rather than completely blocking, IKK-NF-κB signaling seems to be the way to go.

SUMMARY

The success of small-molecule kinase inhibitors in the treatment of cancer has spurred efforts to identify kinase targets for the treatment of RA. Many kinases have been convincingly implicated in the pathogenesis of RA, and many kinase inhibitors have proved efficacious in the treatment of inflammatory arthritis in animals. Few kinase inhibitors, however, have so far made it into clinical development, let alone survived the rigors of a phase II RA clinical trial. This is partly because the therapeutic index of a therapy needs to be higher for a chronic inflammatory disorder, such as RA, than for cancer. The kinase inhibitors approved for the treatment of cancer are not very selective, and inhibition of multiple kinases heightens the risk of adverse effects.

In selecting suitable therapeutic targets for chronic diseases, not only must potential off-target effects be minimized but also target-based toxicities must be rigorously scrutinized. For instance, the experience with p38α inhibitors highlights the importance of appraising the potential effects of kinase inhibition on feedback loops. Furthermore, the identification of several commonly targeted kinases as important regulators of cardiac function underscores the need for careful selection of kinase targets to preclude cardiotoxicity.[91] Finally, caution should be exerted in assigning culpability to a specific kinase on the basis of the effects of small-molecule inhibitors, most of which lack specificity.

Despite these hurdles, the treatment of RA with oral kinase inhibitors seems within reach. Attaining the fine line between therapeutic efficacy and toxicity is crucial and tricky, but it may be possible. Unlike cancer, which is frequently driven by mutations in kinases and thus requires treatment with high doses of kinase inhibitors, inflammatory diseases are driven by aberrant activation of wild-type kinases, against which low doses of inhibitor may be effective. Lower doses of kinase inhibitors should result in greater selectivity and reduced toxicity. Moreover, as recently illustrated for IKK, inhibition of an essential kinase—if not absolute—may be tolerated. Such partial sparing of target kinase activity may well underlie the tolerability of many of the kinase inhibitors tested and should perhaps be an overt goal in the development of new kinase inhibitors. Emergent kinome-profiling technologies are expected to facilitate the discovery of additional kinases involved in RA and the development of more-selective kinase inhibitors. Greater specificity may also be achieved by targeting substrate- or scaffold-protein-specific docking sites on kinases rather than the highly conserved ATP-binding sites, as illustrated by the pepJIP1 and its small-molecule mimic, T-5224. Finally, the burgeoning efforts at biomarker discovery in RA may one day mean that even those kinase inhibitors now relegated to the scrap heap can be used as effective and safe therapy in specific patient subsets.

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