

Signalling platforms that modulate the inflammatory response: new targets for drug development

Christopher. A. McCulloch*, Gregory P. Downey† and Hani El-Gabalawy§

Abstract | Therapeutically controlling inflammation is essential for the clinical management of many high-prevalence human diseases. Drugs that block the pro-inflammatory cytokines tumour-necrosis factor- α and interleukin-1 (IL-1) can improve outcomes for rheumatoid arthritis and other inflammatory diseases but many patients remain refractory to treatment. Here we explore the need for developing new types of anti-inflammatory drugs and the emergence of novel drug targets based on the clustering of IL-1 receptors into multi-protein aggregates associated with cell adhesions. Interference with receptor aggregation into multi-protein complexes effectively abrogates IL-1 signalling. The exploration of the crucial molecules required for receptor clustering, and therefore signal transduction, offers new targets and scope for anti-inflammatory drug development.

Innate and acquired immunity

Innate immune responses are activated by pathogens through ligation of Toll-like receptors expressed on the surface of epithelial cells, neutrophils, macrophages, natural killer cells and dendritic cells. Acquired immune responses are highly specific and develop as a result of antigen processing by antigen-presenting cells with subsequent presentation to T cells.

*CIHR Group in Matrix Dynamics, University of Toronto, Toronto, Canada M5S 3E2.

†Toronto General Hospital Research Institute of the University Health Network, and Division of Respiratory, Department of Medicine, University of Toronto, Toronto, Canada M5G 2C4.

§Department of Medicine, University of Manitoba, Winnipeg, Canada. Correspondence to C.A.M.: e-mail: christopher.mcculloch@utoronto.ca doi:10.1038/nrd2109

Persistent and recurrent episodes of inflammation mediated by aberrant activation of innate and acquired immunity characterize a wide spectrum of idiopathic and infectious chronic inflammatory disorders (BOX 1, TABLE 1). These disorders include systemic autoimmune diseases such as **rheumatoid arthritis**; auto-inflammatory diseases resulting from inherited mutations of single genes that regulate innate immune responses; and high-prevalence disorders such as **atherosclerosis** that exhibit persistent inflammation in specific tissues as an integral part of their pathogenesis and progression. Whether because of mutations in a single gene or because of complex multi-gene–environmental interactions, each of these disorders features a unique and reproducible pattern of tissue inflammation. For example, inflammation primarily targets the synovium in rheumatoid arthritis whereas in **inflammatory bowel disease** the bowel mucosa is the principally affected tissue. In other disorders, such as systemic vasculitis, there is typically widespread tissue inflammation in multiple organs that is due to involvement of the systemic vasculature. Ultimately, independent of the target tissue, persistent inflammation often leads to irreversible tissue damage and loss of organ function. Consequently, early effective control of both systemic and tissue inflammation is the single most important strategy for preventing irreversible organ damage.

Despite major differences in their clinical manifestations, rheumatoid arthritis, **systemic lupus erythematosus** (SLE), inflammatory bowel disease and systemic vasculitis

share a number of pathogenetic features. In particular, these disorders feature a systemic syndrome often referred to as the acute-phase response that is associated with disorders of blood formation and the endocrine system¹. The central role of the cytokines interleukin-1 (IL-1), tumour-necrosis factor- α (TNF α) and IL-6 in mediating the acute-phase response and systemic inflammation is well recognized. However, the role of each of these cytokines in the inflammation detected in specific target tissues is poorly defined. The clinical development of specific and potent inhibitors of TNF α , IL-1 and, more recently, IL-6² has provided an opportunity to gain a better understanding of the role of each cytokine in disease pathogenesis. Several approaches have been developed for the pharmacological regulation of IL-1 and TNF α signals by either receptor blockade, interference with cytokine function, or inhibition of the production, processing and release of the cytokine.

TNF α as an anti-inflammatory target

Targeted anticytokine therapy is now well established in the management of rheumatoid arthritis and Crohn's disease. In rheumatoid arthritis the impressive control of inflammation that can be achieved with three TNF α inhibitors currently approved for clinical use (infliximab (Remicade; Centocor), etanercept (Enbrel; Amgen/Wyeth) and adalimumab (Humira; Abbott)) indicates that TNF α has a crucial role in disease pathogenesis.

Box 1 | Clinical manifestations of chronic inflammatory disorders**Adult onset Still's disease**

Adult variant of Still's disease; similar manifestations as described for systemic onset juvenile idiopathic arthritis below.

Ankylosing spondylitis

Systemic inflammatory disease primarily affecting the spine, and leading to irreversible fusion of spinal joints.

Inflammatory bowel disease

Systemic inflammatory diseases affecting the large and small intestines causing diarrhoea, pain and damage to the intestinal mucosa and wall. The ulcerative colitis variant affects primarily the colon, whereas Crohn's disease affects colon, ileum and occasionally other areas.

Periodontitis

Local inflammatory disease of the gingiva and supporting bone of the teeth that can lead to tooth loss.

Psoriatic arthritis

Arthritis of peripheral joints and/or the spine, associated with psoriasis skin lesions and leading to irreversible joint damage as is seen with rheumatoid arthritis. Although psoriatic arthritis has distinctive features, it can be difficult to differentiate from rheumatoid arthritis, although autoantibodies are usually absent.

Pulmonary fibrosis

An idiopathic inflammatory disorder of the lung parenchyma that leads to irreversible scar formation and reduced gas-exchanging capacity.

Rheumatoid arthritis

Systemic autoimmune disease primarily affecting the peripheral joints and leading to irreversible joint damage and loss of joint function. Rheumatoid arthritis typically associated with the development of specific autoantibodies such as rheumatoid factor and anticitrulline antibodies.

Systemic lupus erythematosus

Systemic autoimmune disease characterized by uncontrolled autoantibody production associated with variable damage to multiple organs including the kidneys, lungs, joints, skin and brain.

Systemic onset juvenile idiopathic arthritis (Still's disease)

A childhood systemic inflammatory disease affecting the joints and several visceral organs, characterized by recurrent fever and rash.

Systemic vasculitis

A group of related systemic inflammatory disorders affecting blood vessels of various sizes with resultant damage to the tissues supplied by the blood vessels. Certain forms of vasculitis can be life-threatening.

Moreover, TNF α inhibitors can arrest erosive articular damage as evaluated by radiography³⁻⁷. This anti-erosive effect is probably related to inhibition of osteoclastogenesis in the periarticular bone, and might be independent of the effect on synovial inflammation⁸. Further, the central role of TNF α in rheumatoid arthritis (and probably other destructive inflammatory arthropathies such as **psoriatic arthritis** and **ankylosing spondylitis**) is supported by animal models, in particular the TNF α transgenic mouse⁹⁻¹⁴. In this well-studied model, human TNF α is overexpressed; the mice subsequently develop a spontaneous destructive arthritis with many of the features of human rheumatoid arthritis. Furthermore, TNF α blockade is highly effective in suppressing the inflammatory response and in preventing the progressive articular damage that is related to the impact of TNF α on osteoclastogenesis¹³.

Despite the generally favourable clinical responses to TNF α inhibition in most cases of rheumatoid arthritis,

a substantial proportion of individuals (~30% in many clinical trials) fail to respond¹⁵. In some cases there is a 'primary' failure in which no response is evident at any point; in other cases there is a 'secondary' failure in which an initially favourable clinical response dissipates after continued use of the agent. Primary and secondary failure of TNF α inhibition probably relate to distinct pathophysiological mechanisms. Notably, patients with rheumatoid arthritis who do not respond to one TNF α inhibitor sometimes respond well to another¹⁵. These observations, combined with the favourable response of patients with Crohn's disease to infliximab (a monoclonal antibody against TNF α) but not etanercept (a soluble TNFR1 receptor fusion protein), suggest that the role of TNF α in rheumatoid arthritis and other chronic inflammatory disorders is complex and is not completely defined. So although the success of TNF α inhibition for treatment of chronic inflammatory diseases has validated specific targeting of pro-inflammatory cytokines, the relative lack of efficacy in a significant number of patients underlines the limitations of targeting one cytokine. Concurrent with the development of TNF α inhibitors, considerable efforts have been devoted to block IL-1 signalling.

Role of interleukin-1 in inflammatory diseases

Like TNF α , IL-1 proteins are potent, pro-inflammatory cytokines¹⁶ capable of inducing multiple signalling cascades that can serve in host defence or, paradoxically, contribute to inflammatory tissue injury¹⁷. IL-1 stimulates the expression of the early response genes *c-fos* and *c-jun* as well as multiple cytokines and inflammatory factors that drive extracellular matrix degradation¹⁸ in rheumatoid arthritis, **pulmonary fibrosis** and periodontal diseases¹⁹⁻²¹. Human clinical trials have demonstrated the efficacy of IL-1 receptor antagonists in ameliorating inflammatory pain and bone resorption in patients with rheumatoid arthritis²². As IL-1 is a downstream mediator of TNF α -induced pathologies¹⁷, the efficacy of blocking TNF α might be due in part to its upstream regulation of IL-1 function.

Inhibition of IL-1 is an effective therapeutic strategy for several autoimmune and auto-inflammatory disorders (TABLE 1), and a number of strategies for interfering with IL-1 signalling are in various stages of development^{23,24}. Therapies aimed at blocking IL-1 function are based on our current knowledge of signalling through IL-1 receptors. The IL-1 receptor (IL-1R) belongs to the IL-1/Toll-like superfamily of receptors that contain, within their cytoplasmic domain, a highly conserved region (TIR domain)^{17,25}. Ligand binding to IL-1R results in activation of complex and interrelated signalling cascades²⁶. There are two membrane-bound IL-1 receptors, type I and type II (IL-1RI and IL-1RII)^{27,28}. Both are expressed by various cell types, including fibroblasts, synovocytes and endothelial cells. IL-1RI is capable of generating a signal whereas IL-1RII acts as a decoy receptor that acts as a trap for IL-1. By blocking interaction with the IL-1 receptor, IL-1RII downregulates the response to IL-1²⁹, thereby acting as a molecular trap for the agonist and downregulating the response to IL-1.

Synovium

The thin layer of connective tissue that forms the inner lining of the joint cavity, which primarily serves to maintain the health of the cartilage.

Acute-phase response

A stereotyped syndrome characterized by the presence of constitutional symptoms such as fatigue and weight loss, elevation in acute-phase proteins such as C-reactive protein and a host of haematological and endocrine changes.

Table 1 | Inflammatory diseases that are potentially treatable with anti-inflammatories

Disease	Prevalence (per 100,000)	Single gene mutation	Site of inflammation	Response to TNF α inhibition	Response to IL-1 inhibition	Therapeutic challenges
Rheumatoid arthritis	500–1,000	No	Synovium, systemic	+++	++	Disease heterogeneity, 30% non-responders to TNF inhibitors
Crohn's disease	100–200	No	Intestines, systemic	+++	—	Remitting, relapsing disease
Vasculitis	200–300	No	Multiple organs, skin, musculo-skeletal	+	—	Wide spectrum of manifestations; some life threatening
SOJIA	50–1000 children	No	Synovium, skin, systemic	+	+++	Severe inflammation in very young children
AOSD	1–2	No	Synovium, skin, systemic	+	+++	Difficult to diagnose
Auto-inflammatory diseases						
FMF	Rare	Yes	Synovium, systemic	+	—	Episodic
TRAPS	Rare	Yes	Synovium, systemic	+++	—	Episodic
NOMID	Rare	Yes	CNS, systemic	—	+++	Episodic
Muckle-Wells	Rare	Yes	Skin, systemic	—	+++	Episodic
FCAS	Rare	Yes	—	—	—	Episodic
PAPA	Rare	Yes	Oropharynx, systemic	—	—	Episodic
Behcet's syndrome	2–30	No	Synovium, skin, eyes, CNS	+	+	Can have severe CNS and eye inflammation
Other						
COPD	3,000–10,000	No	Lungs	—	—	—
Osteoarthritis	5,000–10,000	No	Synovium	—	—	Phase II trial data pending; intra-articular safety
Atherosclerosis	1,000–2,000	No	Vascular endothelium	—	—	—
Periodontitis	5,000–10,000	No	Gingiva/periodontium	—	—	—

A spectrum of chronic and recurrent inflammatory disorders and their prevalence is shown. A summary of the currently available clinical experience with inhibitors of TNF α and interleukin-1 (IL-1) is indicated, where available, for each condition. Typical clinical responses are indicated at follows: +++, good to excellent responses in most patients treated; ++, good to excellent response in some patients; +, modest response in most patients treated with occasional good response. AOSD, adult-onset Still's disease; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; SOJIA, systemic-onset juvenile rheumatoid arthritis; TRAPS, tumour-necrosis factor receptor-associated periodic syndrome; NOMID, neonatal onset multisystem inflammatory disease; PAPA syndrome, pyogenic arthritis, pyoderma gangrenosum, and acne syndrome.

Erosive articular damage

The development of defects in the cartilage and bone adjacent to the joint cavity caused by chronic inflammation of the synovium lining the joint.

Osteoclastogenesis

The process of generating bone-resorbing multinucleated cells from blood-forming precursor cells that is mediated by the sequential action of specific cytokines and growth factors.

Periarticular bone

Bone that is subjacent to the cartilage-covered, load-bearing surfaces of joints.

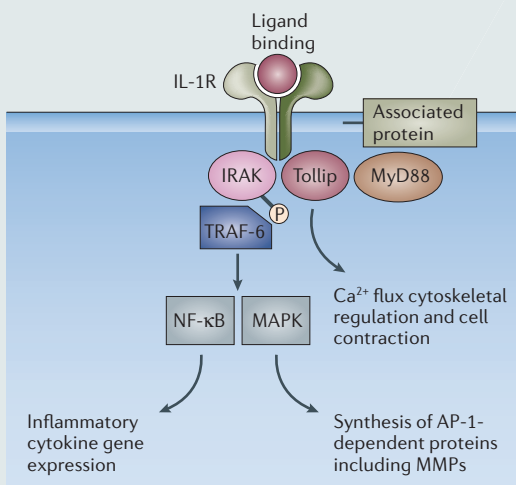
Following ligand binding, the IL-1R-associated protein is recruited to IL-1R₁³⁰. Subsequently, a complex is formed including IL-1R-associated kinases (**IRAK1** and **IRAK2**), **TOLLIP** (Toll-interacting protein) and the adapter protein **MYD88** (myeloid differentiation primary response gene (88))^{31,32}. IRAK is rapidly phosphorylated and associates with **TRAF6** (TNF receptor-associated factor 6)³¹; this association is necessary for downstream IL-1-induced activation of nuclear factor- κ B (NF- κ B). Ligand binding to IL-1 R₁ can activate multiple signalling events^{33–39} (BOX 2) that ultimately lead to expression of genes that mediate inflammation and, frequently, tissue destruction.

Current IL-1 inhibitors

Several approaches for inhibiting IL-1 signalling activity have either been developed or are in the process of development; a detailed review summarizing data

from various clinical trials has been published²³. These approaches include the development of small-molecule inhibitors of IL-1 that can affect the production, processing or release of IL-1 β , including blockade of the IL-1 converting enzyme (ICE, also known as **caspase 1**). The latter approach prevents the processing of the IL-1 β precursor into an active cytokine. Neutralizing antibodies to IL-1 β or IL-1 receptors, and IL-1 receptor antagonists, are also under development for clinical applications¹⁷. Newer approaches also include inhibitors of processes that regulate IL-1 maturation and release. For example, there is growing evidence that the plasma membrane receptor for extracellular ATP, the P2X₇ receptor, is a crucial regulator of both IL-1 maturation and release. Current work is now focused on identifying drugs that block P2X₇ receptor function and that will subsequently inhibit triggering of IL-1 maturation and exteriorization⁴⁰.

Box 2 | IL-1-generated signals



Following binding of interleukin-1 (IL-1) to the IL-1 receptor 1 (IL-1R1), multiple signals are generated which include the following: phosphorylation of multiple kinases and receptor-associated proteins³³; induction of mitogen-activated protein kinase (MAPK) cascades^{34,35}; Ca²⁺ flux, cell contraction and actin cytoskeleton reorganization^{36,37}; translocation of signalling molecules to the nucleus³⁸; and synthesis of AP-1-dependent proteins via the transcription of the immediate early genes *c-fos* and *c-jun*³⁹.

IRAK, IL-1R-associated kinase; MMP, matrix metalloproteinase; MYD88, myeloid differentiation primary response gene; NF-κB, nuclear factor-κB; TOLLIP, Toll-interacting protein; TRAF6, TNF receptor-associated factor 6.

Post-translational processing of IL-1β is associated with activation of caspase 1. Discrete types of diarylsulphonylureas can inhibit these processes; these and other drugs with similar activities are known collectively as cytokine-release inhibitory drugs (CRIDs). CRIDs can arrest activated macrophages and monocytes in such a manner that caspase 1 is not activated, but their mechanism of action and molecular targets are poorly defined. One potential target is **glutathione S-transferase omega 1-1** (REF. 41). Additional approaches for blockade of IL-1 activity include the utilization of IL-1 receptor accessory protein (IL-1RacP) and IL-1 receptor antagonist (IL-1RA). Recent data comparing these approaches in collagen-induced arthritis in mice indicate that IL-1RacP can improve experimental arthritis without affecting T-cell immunity, whereas IL-1RA has an obvious impact on T-cell function in this model. These different approaches for mediating receptor competition show that biological outcomes downstream of IL-1 receptor blockade are not consistent between agents and might suggest an explanation for the different mode of IL-1 antagonism in comparison with IL-1RA⁴².

Despite the wide range of possible IL-1-blocking agents, the only clinically approved agent for specific blockade of IL-1 signalling is anakinra (Kineret; Amgen). This agent is a recombinant version of the naturally occurring IL-1 inhibitor IL-1RA, and is

administered by daily subcutaneous injection. Anakinra has an excellent safety record and low reported incidence of infection or malignancy⁴³. By contrast, higher concentrations of drugs that block TNFα function are associated with increased risk of *Mycobacterium tuberculosis* infection⁴⁴, an effect that might be due to reduced expression of interferon-γ (IFNγ)⁴⁵. Indeed, the increased incidence of opportunistic infections following treatment with TNFα indicates a more global impairment of host defence mechanisms that has not been reported with anakinra.

Clinical trials with anakinra clearly demonstrate the benefit of this agent in ameliorating synovial and systemic inflammation in rheumatoid arthritis while also retarding the progression of articular damage^{22,46–48}. These observations are consistent with findings in animal models of rheumatoid arthritis^{42,49}. Yet the overall clinical experience suggests that anakinra is a less effective anti-rheumatic agent than anti-TNFα drugs. The short half-life of anakinra⁵⁰ and the requirement of high, continuous serum levels for treatment efficacy could account for the relatively modest improvement in patient responses compared with anti-TNFα-based drugs: many cell types express relatively high numbers of IL-1 receptors, and so systemic IL-1 levels need to be well controlled by therapeutics otherwise IL-1 receptors will be activated. Yet there must be other factors that underlie the relatively more efficacious results with TNFα blockers compared with the IL-1 blockers. As described above, the different T-cell responses to blockade by IL-1RacP and IL-1RA⁴² indicates that the relative numbers of functional receptors might not be the limiting factor for the generation of IL-1 signals. Clinically, a potential approach to overcome inherent limitations of IL-1 receptor blockade is simply to use direct intra-articular injection of anakinra into human knee joints for management of osteoarthritis⁵¹. This approach increases local drug concentrations, prolongs treatment effects and restricts the drug to affected sites. Although the intra-articular injection approach is apparently safe, randomized controlled trials to establish treatment efficacy have not been reported.

In contrast to the treatment of rheumatoid arthritis, recent clinical experience with anakinra for the management of other systemic inflammatory disorders has been more impressive. In particular, anakinra is remarkably effective in controlling the clinical manifestations seen in two related disorders: systemic onset juvenile idiopathic arthritis⁵² and adult onset Still's disease⁵³. Notably, the exaggerated production of IL-1 by stimulated peripheral blood mononuclear cells is consistent with the notion that defects in IL-1 regulation are central to the pathogenesis of systemic onset juvenile idiopathic arthritis⁵².

Similar observations are emerging from clinical studies of the auto-inflammatory syndromes. In these disorders, IL-1 is aberrantly regulated by the inflammasome in cells of the innate immune system^{54,55}. Indeed, assembly of the inflammasome is a crucial part of the innate immune response. The adaptor protein ASC (apoptosis-associated speck-like protein) is essential for inflammasome function and binds directly to caspase 1⁵⁶, but the triggers of this interaction are less clear.

Inflammasome

A cytosolic complex of proteins that activates caspase 1 to process pro-inflammatory cytokines such as IL-1β and IL-18.

ASC also interacts with the adaptor cryopyrin (encoded by the *CIAS1* gene). A syndrome designated as neonatal onset multi-system inflammatory disease (NOMID) is characterized by severe multi-system inflammation relating to mutations in the *CIAS1* gene (also known as *NALP3*). Cryopyrin activates ICE (caspase 1), which in turn activates IL-1 β , resulting in uncontrolled inflammatory episodes. The efficacy of anakinra in controlling this syndrome has recently been demonstrated⁵⁷, and also provided benefit in a 5-year-old boy with a diagnosis of NOMID with neonatal onset of urticaria-like rash, chronic fever, systemic inflammation, hepatosplenomegaly and chronic inflammation of the central nervous system⁵⁸.

In view of the crucial role of the inflammasome in the regulation of IL-1 production, the components of this complex have become appealing targets for drug development. In particular, ICE/caspase 1 has been the focus for small-molecule drug development. Pralnacasan (Aventis/Vertex) is an oral ICE inhibitor that is undergoing clinical trials in rheumatoid arthritis and several other inflammatory disorders. This agent, which inhibits lipopolysaccharide-stimulated IL-1 β production by peripheral blood monocytes, was shown to be effective in controlling articular damage in two murine osteoarthritis models⁵⁹ and was effective in controlling inflammation in a murine model of inflammatory bowel disease⁶⁰. The results of a Phase IIa clinical trial of 285 rheumatoid arthritis patients have been published in abstract form⁶¹. The available data from this 12-week, double-blind, randomized, placebo-controlled trial suggested a dose-dependent clinical response associated with a reduction in acute-phase reactants. The drug was safe and well tolerated. A Phase IIb clinical trial initiated in 2003 was terminated prematurely because of animal data that indicated that the agent caused hepatic toxicity, although this had not been observed in the human studies. Clinical trials in osteoarthritis and psoriasis have been initiated, but to date the results of these studies have not been published.

A particularly promising approach that targets IL-1 signalling is a recombinant fusion protein to trap IL-1⁶². The protein contains extracellular binding motifs of IL-1 receptor 1 and IL-1RA protein in one chain; two chains are coupled to the Fc fraction of human immunoglobulin G to form a dimeric protein. IL-1 is bound between the two arms of the trap with very high affinity ($K_d < 1.5$ pM), effectively blocking IL-1 β from binding to IL-1 receptors. Compared with other agents that neutralize IL-1, the IL-1 trap has >60-fold higher affinity against IL-1 α and IL-1 β than the IL-1 receptor or antibody to IL-1. Phase Ib studies of subcutaneously administered drug indicated good safety and patient tolerance of the drug⁶³, no increase of infections and a measurable reduction of inflammation, C-reactive protein and scores on the American College of Rheumatology scales. However, statistically significant differences between placebo and drug in the *a priori* endpoints of intent-to-treat were not achieved and no further data have been forthcoming for the treatment of rheumatoid arthritis.

Based on the data reviewed above and elsewhere^{17,23}, there is a need for the development of novel therapeutic approaches that target IL-1 signalling. This need relates to inherent pharmacological problems associated with the use of biological agents that inhibit cytokine action through receptor blockade, including tissue distribution, protein degradation, development of immune responses to administered proteins and changes in the cell-surface expression of the relevant receptors.

In addition to current approaches that block IL-1 production or interfere with IL-1-receptor interactions, other components of the IL-1 signalling pathway might also provide promising therapeutic targets. Further, specific cell lineages in many types of chronic inflammatory lesions are induced by IL-1 to produce matrix-destructive molecules that are responsible for much of the irreversible tissue damage in inflammatory diseases. For example, in rheumatoid arthritis, cells of the mesenchymal lineage produce effectors of tissue destruction, such as matrix metalloproteinases and reactive oxygen species, when stimulated by IL-1¹⁷. In view of the need to develop new IL-1-based therapies that target specific cell populations, we consider below novel approaches to block IL-1 signalling in mesenchymal cells based on the restrictions imposed by macromolecular adhesion complexes on signal transduction.

IL-1-signalling and focal adhesions

Spatial sequestration of interacting molecules is emerging as a pivotal regulatory locus for signal transduction. Multi-molecular protein complexes on the cytosolic face of the plasma membrane have been implicated in a wide range of signalling systems including epidermal growth factor (EGF)⁶⁴, constitutively photomorphogenic 9 (COP9)⁶⁵, TNF α ⁶⁶ and endothelin⁵¹. Adapter proteins can help to localize signalling molecules in time and space, thereby increasing the efficiency and specificity of the molecular interactions required for signal transduction. For example, the protein tyrosine phosphatase SHP2 (Src homology phosphatase 2) regulates EGF signalling through the adaptor function of GAB1⁶⁷. More complex and larger macromolecular complexes have been associated with the IL-1 signalling pathway. Immunohistochemistry and ¹²⁵I-labelling experiments^{36,68,69} have established a tight spatial relationship between IL-1 receptors and restricted adhesive domains of mesenchymal cells designated as focal adhesions. In fibroblasts, synovocytes and chondrocytes the localization of IL-1 receptors to focal adhesions is crucial for IL-1 signal transduction. Notably, tyrosine phosphorylation and activation of the focal adhesion kinase (FAK), a key molecule in IL-1 signal transduction, does not occur in the absence of focal adhesion complexes and IL-1-induced Ca²⁺ fluxes are not propagated without activation of focal adhesion kinase⁶⁸.

This focal adhesion 'restriction' (that is, the *selective* activation of pathways under conditions in which focal adhesions are formed) illustrates the notion that propagation and regulation of IL-1 signalling pathways are regulated by the formation of signalling platforms comprising proteins that interact via specific cellular

Mesenchymal

The part of the embryonic mesoderm from which connective tissue, bone, cartilage, and the circulatory and lymphatic systems develop.

Protein tyrosine phosphatase

A group of enzymes that remove phosphates from tyrosine residues by hydrolysis.

Focal adhesions

Actin-enriched anchorage sites of adherent cells where there is close apposition of the plasma membrane to the substratum. Focal adhesions are enriched in actin-binding proteins and molecules associated with signalling processes.

Fibroblast

Ubiquitous cells of connective tissue that synthesize and remodel collagen and other extracellular matrix proteins.

Synovocyte

Cells of soft connective tissues that line the joints and which upon activation can contribute to the degradation of joint tissues.

Chondrocyte

A connective tissue cell that resides in a lacuna within the cartilage matrix.

Table 2 | Scaffold and signalling molecules associated with focal adhesions as potential therapeutic targets

Category	Specific examples	Functions and potential drug targets	Therapeutic possibilities
Serine threonine kinases	PAK, integrin-linked kinase, protein kinase C, ERK and JNK	Link to downstream signalling pathways regulating inflammatory gene expression	Small-molecule inhibitors, cell-permeant peptides and siRNA
Exchange factors	PIK, GIT1, Sos	Link to signalling pathways regulating cytoskeletal alterations, FAC assembly and generation of IL-1 signalling	Small-molecule inhibitors, cell-permeant peptides and siRNA
Protein serine threonine phosphatases	Protein phosphatase1 δ	Link to signal transduction pathways controlling inflammation; control maturation of FAC	Small-molecule inhibitors, cell-permeant peptides and siRNA
Small GTPases	Rho A, Rac 1, Ras	Signals for cytoskeletal remodeling	ROC kinase inhibitor (for example, fasudil)
Protein tyrosine kinases	FAK, Src, Fyn, Yes, Abl, Csk, PYK2, LIM kinase	Regulate assembly and disassembly of FAC	Small-molecule inhibitors, cell-permeant peptides and siRNA
Protein tyrosine phosphatases	SHP2, low-molecular-weight-PTP, PTP α , PTPe, PTP-PEST, PTP1B, SHP1, LAR	Regulate maturation of FA, adaptor function to recruit signalling molecules	Small-molecule inhibitors, cell-permeant peptides, siRNA and oxidants
Lipid kinases	PI5K, PI3K	Link to signal transduction pathways regulating inflammation; control maturation of FAC	Small-molecule inhibitors, cell-permeant peptides and siRNA
Phospholipases	Phospholipase C γ	Regulate calcium fluxes via Ins(1,4,5)P $_3$	Small-molecule inhibitors, cell-permeant peptides and siRNA
Adaptor molecules	Gab1, Gab2, Grb2	Link proximal signalling pathways to ERK	Small-molecule inhibitors, cell-permeant peptides and siRNA
Structural/scaffold molecules	Actin, vinculin, talin, actinin, paxillin, tubulin, mDia 1/2	Facilitate and stabilize and multi-molecular signalling platforms	
Receptors	IL-1R, integrins, syndecans	Clustering of receptors brings molecules into proximity and facilitates signalling	Receptor blocking, ligand sequestration (traps); focal-adhesion-dispersing peptides

ERK, extracellular-regulated kinase; FAC, focal adhesion complex; FAK, focal adhesion kinase; GIT1, G protein-coupled receptor kinase interactor 1; IL-1, interleukin-1; IL-1R, IL-1 receptor; Ins(1,4,5)P $_3$, inositol-1,4,5-trisphosphate; LAR, leukocyte common antigen related; JNK, C-JUN-amino-terminal kinase; PAK, p21-activated kinase; PEST, proteolytic signal sequences enriched with proline (P), glutamic acid (E), serine (S) and threonine (T) residues; PI3K, phosphatidylinositol 3-kinase; PI5K, phosphatidylinositol 5-kinase; PIK, PAK-interacting exchange factor; PTP, protein tyrosine phosphatase; PYK2, proline-rich tyrosine kinase 2; SHP, Src homology phosphatase; siRNA, small interfering RNA.

domains. This organization serves to ‘focus’ or direct the signals to specific pathways that lead to inflammatory cellular responses. At a macro-molecular level, these signalling complexes are held together in a spatially confined manner by scaffolding proteins including protein tyrosine phosphatases and actin filaments that are important components of focal adhesions. Below we describe the composition of focal adhesions in the context of IL-1 signalling and consider how these adhesive domains could provide attractive targets for the design of new drugs that target the IL-1 signalling pathway (FIG. 1).

Focal adhesions as signalling complexes

Focal adhesions are found in many types of mesenchymal cells, including fibroblasts, endothelial cells, synovocytes and chondrocytes⁷⁰. Although once thought to be strictly structural adhesive complexes, focal adhesions are increasingly recognized for their role in signal transduction^{71,72}. Indeed, these complex structures are now known to

form multimeric signalling complexes that orchestrate essential aspects of cell behaviour including cell shape, motility, proliferation, apoptosis and responses to environmental cues such as physical forces, growth factors and inflammatory stimuli⁷³. In addition to integrins and structural cytoskeletal proteins such as vinculin, talin, tensin, paxillin, zyxin and α -actinin, focal adhesions contain a diverse array of signalling molecules (>50 to date and counting), including protein kinases and phosphatases, small GTPases and associated regulatory molecules, and adaptor molecules that mediate key protein–protein interactions (TABLE 2)⁷⁴. Some of these focal adhesion molecules are known to be directly involved in IL-1 signalling because engagement of IL-1R by IL-1 β leads to phosphorylation of the scaffold protein talin³³ and focal adhesion kinase⁶⁸. The repertoire of focal adhesion proteins that are involved in IL-1 signalling is dependent on the extent of focal adhesion maturation following initial cell attachment. A more global view of the large number of potential signalling regulatory

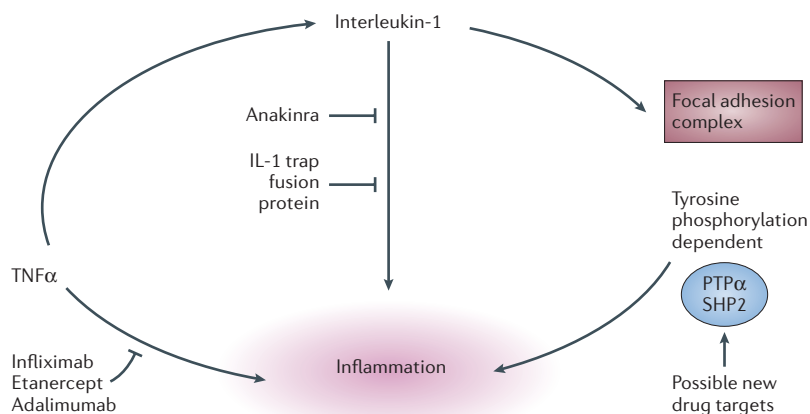


Figure 1 | Sites of action of existing and novel therapeutics for the treatment of inflammation. Existing therapies include the TNF α inhibitors infliximab, etanercept and adalimumab, and the IL-1R antagonist anakinra. Studies using IL-1 trap are currently in clinical development, whilst molecules that regulate interleukin via focal adhesion complexes, such as SHP2, represent novel therapeutic targets. PTP, protein tyrosine phosphatase; SHP2, Src homology phosphatase 2.

molecules in focal adhesions (TABLE 2) indicates wide scope for pharmacological target discovery both in cancer and, most notably, inflammation.

Focal adhesion maturation

Focal adhesions often mature through a series of stages (focal contacts, focal adhesions and fibrillar adhesions), each with a distinctive appearance and molecular composition. In the absence of exogenous stimuli, focal adhesions develop and mature slowly over many hours. Exogenous stimuli can profoundly modulate this process; exposure to growth factors such as platelet-derived growth factor (PDGF), cytokines such as IL-1 β , and mechanical forces can promote maturation and dynamic remodelling of focal adhesions. Many of these responses are regulated by tyrosine phosphorylation-dependent events that are crucial to the formation, maturation and dynamic remodelling of focal adhesions as well as modulation of downstream signalling pathways⁷⁵. In the context of drug discovery for blockade of IL-1 signals, prevention of focal adhesion maturation with peptides that disperse focal adhesions⁷⁶ blocks IL-1 signalling that leads to extracellular signal-regulated kinase (ERK) activation⁷⁷. These data illustrate the potential for using cell adhesions and cell adhesion-related proteins as targets for novel therapeutics.

Phosphorylation regulates focal adhesions

Focal adhesions contain numerous tyrosine phosphorylated proteins including paxillin, focal adhesion kinase and Src family kinases. The latter have pivotal and multifaceted roles in focal adhesion formation and maturation^{73,78-80} (FIG. 2). In the context of IL-1 signalling, tyrosine phosphorylation of FAK in response to IL-1 is required for signal transduction in fibroblasts⁶⁸, underscoring the importance of tyrosine phosphorylation in regulation of IL-1 signal transduction. Indeed, tyrosine phosphorylation is pivotal in the formation, maturation

and dynamic remodelling of focal adhesions as well as in the modulation of many downstream signalling pathways⁷³ including IL-1^{68,81}. As noted above, focal adhesions contain numerous tyrosine-phosphorylated proteins as determined by immunofluorescence microscopy and western analysis of focal-adhesion-associated proteins⁷⁸. Early studies using pharmacological inhibitors provided indirect evidence for the importance of tyrosine kinases in focal adhesion formation⁷¹. The use of dynamic quantitative fluorescence microscopy revealed that focal adhesion assembly was accompanied by a rapid increase in the local density of several tyrosine-phosphorylated proteins, including vinculin, paxillin and FAK⁷⁹. Subsequently, the multifaceted roles of specific tyrosine kinases, including FAK and the Src family kinases Src, Fyn and Yes, in focal adhesion formation and maturation were defined^{80,82}. For example, FAK is now appreciated to have a dual role in this regard: at the earliest stages of focal adhesion formation during integrin clustering, autophosphorylation of Y397 serves to recruit Src family kinases, which leads to additional phosphorylation of FAK, recruitment of additional signalling molecules and promotion of focal adhesion assembly⁸³. Conversely, phosphorylation of FAK at the C-terminal Y925 leads to disassociation of FAK from focal adhesions, thereby promoting the disassembly of focal adhesions and turnover and downregulation of focal adhesion-dependent signalling pathways⁸⁴. Similarly, Src has dual functions. At early stages Src is involved with focal adhesion assembly mediated via its adaptor function. By contrast, at later stages its kinase activity mediates phosphorylation of FAK on Y925, which promotes focal adhesion disassembly and turnover during cell migration. The importance of these dynamic and reversible tyrosine phosphorylation events in focal adhesion formation, remodelling and signalling illustrate the central role of tyrosine kinases and protein tyrosine phosphatases (PTP) in regulating crucial signalling processes.

PTPs and focal adhesions

Recent studies have documented an important role for PTPs in regulating the maturation of focal adhesions and focal adhesion-dependent signalling. The maturation of focal adhesions has a significant effect on IL-1 signalling because cells with immature focal adhesions do not respond to IL-1^{75,85}. Notably, the tyrosine phosphorylation state of a substrate reflects the balance of activities of protein tyrosine kinases and PTPs, and this balance affects IL-1 signalling, in part through regulation of focal adhesion maturation.

PTPs selectively remove phosphate groups from tyrosine residues and can negatively or positively regulate signalling pathways. Recent estimates suggest that the human genome contains at least 107 PTP genes, comparable to the number and variety of protein tyrosine kinases⁸⁶; however, comparatively less is known about the specific functions of PTPs. Early studies using pharmacological inhibitors of PTP such as pervanadate and phenylarsine oxide documented that these compounds induced dynamic and complex alterations in focal adhesions, providing preliminary evidence of a role for PTP

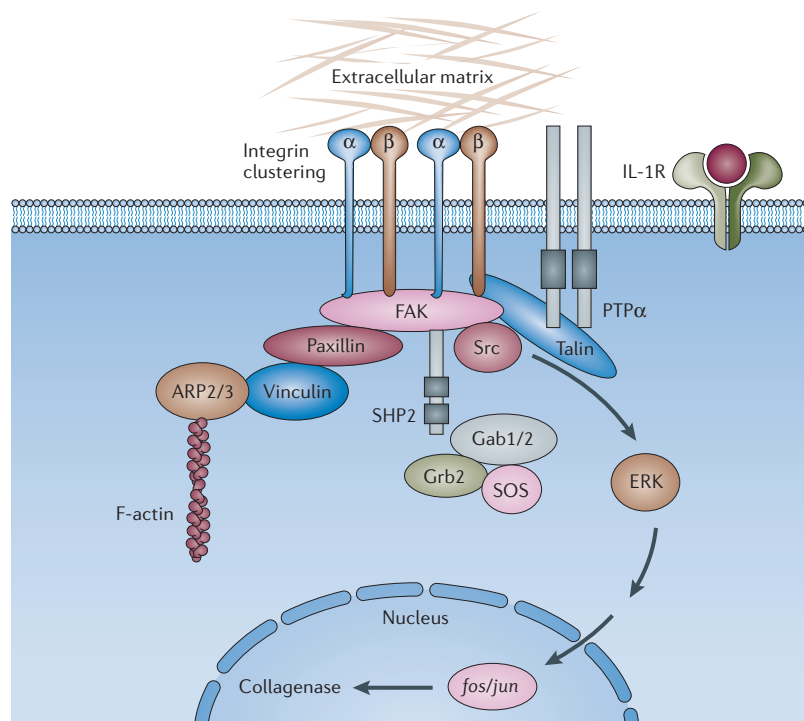


Figure 2 | Focal adhesion maturation. Following integrin clustering in focal adhesions in response to ligation of extracellular matrix, interleukin-1 (IL-1) receptors (IL-1R) and a large number of actin-binding proteins and adaptor proteins are sequentially recruited in fibroblasts, chondrocytes and synoviocytes. The recruitment of receptor and adaptor proteins enables IL-1 signalling to extracellular regulated kinase (ERK) and the generation of matrix-destructive mediators such as collagenase. ARP, actin related protein; FAK, focal adhesion kinase; PTP, protein tyrosine phosphatase.

in these events⁸⁷. Furthermore, several PTPs physically associate with focal adhesions and have emerged as potential modulators of focal adhesion maturation and cytoskeletal organization and signalling. Additional and more definitive evidence for the role of specific PTPs in the regulation of focal adhesions was provided using molecular approaches to manipulate phosphatase gene expression, including expression of dominant-negative mutant phosphatases, transgenic and knockout technology, and live fluorescence imaging using GFP-tagged fusion protein sensors. Selected aspects of these studies will be discussed below, focusing on the role of PTPs in focal adhesion dynamics and signalling.

Categories and structure of PTPs

PTPs can be divided into two broad categories: classical phosphotyrosine-specific phosphatases and dual-specificity phosphatases^{86,88,89}. The former can be further subdivided into receptor-like and non-receptor-like (cytosolic) PTPs. The receptor-like PTPs comprise a membrane-spanning domain, an extracellular domain of variable size that frequently contains structural domains, and a cytosolic domain containing one or two catalytic domains (although only one of these is usually catalytically active)⁹⁰. Most cytosolic PTPs have a multi-domain structure comprising the conserved catalytic domain and additional regulatory or targeting/binding modules such as SH2, PDZ, FERM⁹¹ (Protein 4.1, ezrin,

radixin, moesin domain) or proline-rich domains. Subcellular targeting of PTP is of particular importance and directs the catalytic domain to precise locations, often in the context of multimeric protein complexes designated as *signalsomes* within the cell in proximity to their substrates and regulatory molecules such as inositol phospholipids⁹⁰. Members from each of these PTP groups are involved in focal adhesion dynamics and focal adhesion-dependent signalling.

SHP2, focal adhesions and IL-1 signalling

SHP2 (Src homology phosphatase 2) is involved in several aspects of focal adhesion dynamics and IL-1 signalling. SHP2 is recruited to focal adhesions as a result of integrin engagement^{92,93}. SHP2^{-/-} murine embryonic fibroblasts have increased numbers of focal adhesions and actin stress fibres that are associated with diminished spreading and motility, similar to FAK-deficient cells^{94,95}. Expression of catalytically inactive SHP2 resulted in increased formation of actin stress fibres and focal adhesions, and blocked cellular responses to hepatocyte growth factor/scatter factor⁹⁶. The effects of SHP2 on cytoskeletal organization, focal adhesion dynamics and cell motility are mediated in part via regulation of Rho GTPases^{97,98}. In the context of an inflammatory milieu, SHP2 is recruited to focal adhesions in response to IL-1 β stimulation in fibroblasts and regulates maturation of focal adhesions^{85,99}. SHP2 regulates the kinetics and magnitude of ERK activation and maturation of focal adhesions in response to IL-1, processes that are dependent in part on the adaptor function involving phosphorylation of Y542⁸⁵. SHP2 is also crucial for IL-1-induced phosphorylation of PLC γ and enhances IL-1-induced Ca²⁺ release from the endoplasmic reticulum¹⁰⁰ (FIG. 3). Ca²⁺ release is essential for IL-1 signalling to ERK and downstream pathways that mediate matrix destruction¹⁰¹.

To begin to ascertain the mechanisms by which SHP2 modulates FAC dynamics and signalling, and to look for potential drug targets in focal adhesions, we undertook a proteomic approach using mass spectrometry. Signalling proteins that were present in focal adhesions and/or that were recruited to focal adhesions in an IL-1-dependent manner were surveyed. Collagen-coated magnetic beads to generate focal adhesion-like structures were utilized⁹⁹ and the proteins binding to these beads were analysed. This technique yielded several potentially relevant binding partners including PTP α , PTEN and B23 (nucleophosmin), which might be involved in IL-1 signalling and which could suggest useful targets for drug development.

PTP α . PTP α , a member of the receptor-like PTP family, also associates with focal adhesions¹⁰². During the early phases of cell spreading on fibronectin and vitronectin, PTP α co-localizes and physically associates with α_v -integrins at the leading edge of the cell¹⁰³. During these events, PTP α is responsible for activation of the Src family kinases Src and Fyn via dephosphorylation of C-terminal regulatory tyrosine (Y527 in Src), which is required for the formation of focal

SH2 (Src homology 2) domain
A conserved sequence of amino acids originally identified in the tyrosine kinase Src that mediates binding to tyrosine residues in target proteins.

Signalosomes
Multimeric protein complexes comprising various signalling molecules that by virtue of their spatial clustering enhance signal transduction¹³⁸.

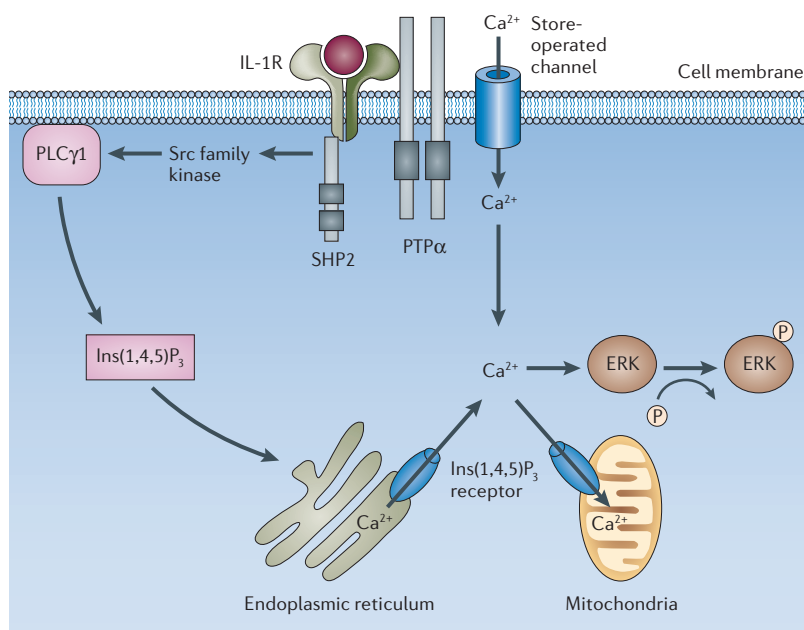


Figure 3 | Interleukin-1-mediated calcium signalling. The recruitment of protein tyrosine phosphatases (PTPs) such as Src homology phosphatase 2 (SHP2) and PTP α into focal adhesions, along with the IL-1 signalling receptor (IL-1R), enables signalling through Src family kinases (SFK) and calcium release from the endoplasmic reticulum. The calcium release from ER stores is crucial for signalling to extracellular-regulated kinase (ERK) and downstream pathways.

adhesions. PTP α ^{-/-} fibroblasts demonstrate reduced spreading on fibronectin, increased numbers of focal adhesions, decreased tyrosine phosphorylation of FAK and the docking protein p130Cas, and attenuated ERK activation in response to adhesion and spreading¹⁰⁴. As PTP α is found in focal adhesions and can physically associate with SHP2, we further investigated the functional significance of PTP α . Human gingival fibroblasts were studied as these cells express high levels of IL-1 type 1 signalling receptors that are concentrated in focal adhesions⁶⁹. IL-1 β induced phosphorylation of PTP α on Y789 in a time-dependent manner that correlated temporally with focal adhesion maturation as defined by morphology, enrichment with paxillin, α -actinin and α -smooth muscle actin, and recruitment of PTP α into focal adhesions (M. Herrera Abreu, preliminary data). Notably, IL-1-induced ERK activation was diminished in PTP α ^{-/-} compared with PTP α ^{+/+} fibroblasts. We also observed that PTP α can physically interact with SHP2. These observations indicate that PTP α modulates IL-1 β -induced maturation of focal adhesions and downstream signalling, possibly by a mechanism involving direct or indirect regulation of SHP2 by PTP α . In summary, SHP2 and PTP α have important roles in focal adhesion dynamics and signalling in the context of IL-1. As both the structure and function of SHP2 (the N-terminal domain contains two SH2 domains) and PTP α (the extracellular and cytosolic portions have unique binding domains) differ, which implies unique groups of interacting proteins, it is anticipated that these two PTPs regulate IL-1 signalling distinctively.

Interleukin-1 and calcium signals

The focal adhesion-dependent rise of $[Ca^{2+}]_i$ in response to IL-1^{68,77} and its dependence on SHP2¹⁰⁰ provide functional links between IL-1 signalling, FAC and tyrosine phosphatases. IL-1 signalling to ERK does not occur if Ca^{2+} fluxes are blocked¹⁰¹. Conversely, focal-adhesion restriction of ERK activation by IL-1 can be overcome by the use of Ca^{2+} ionophores¹⁰¹ to artificially increase $[Ca^{2+}]_i$. The potential importance of Ca^{2+} fluxes in IL-1 signalling is highlighted by the observation that alterations of $[Ca^{2+}]_i$ are important regulatory signals that control the transcription of genes including *c-FOS*¹⁰⁵. IL-1-generated $[Ca^{2+}]_i$ fluxes are dependent on intact actin filaments inserting into focal adhesions⁶⁸, and the capacity of IL-1 to regulate early-response genes such as *c-fos* might rely on physical proximity of intracellular Ca^{2+} stores with membrane-associated FAC proteins, as seen in kidney fibroblasts¹⁰⁶.

How might PTPs be important in mediating these signalling processes? SHP2 in FAC is particularly important in mediating the endoplasmic reticulum–focal adhesion interactions that are required for IL-1 signalling¹⁰⁰. Conceivably, focal adhesions restrict IL-1-induced Ca^{2+} signalling through physical interactions with SHP2 in focal adhesions and the Ins(1,4,5)P₃ (inositol-1,4,5-trisphosphate) receptor in the endoplasmic reticulum. Notably, the Ins(1,4,5)P₃ receptor is both a Ca^{2+} release channel in the endoplasmic reticulum as well as a multi-functional adaptor protein that binds tyrosine phosphatases¹⁰⁷. Furthermore, the Ins(1,4,5)P₃ receptor can be regulated by tyrosine phosphatases¹⁰⁷. Accordingly, SHP2, and possibly other PTPs with adaptor functions, might interact with the Ins(1,4,5)P₃ receptor to mediate focal adhesion-restricted IL-1 signalling.

PTPs evidently have diverse and fundamental roles in the regulation of focal adhesion dynamics and the assembly of multi-molecular signalling platforms that mediate the inflammatory response to IL-1. PTPs modulate signalling pathways that are involved in inflammatory responses¹⁰⁸, including responses to IL-1. The data on IL-1-induced $[Ca^{2+}]_i$ fluxes and activation of focal adhesion and cytoskeletal-associated signalling molecules by Ca^{2+} suggest that PTPs in focal adhesions create a structural context in the cell that regulates the IL-1 signal. These signalling platforms could provide a mechanism for signal termination in which altering the affinity of PTPs for their cognate binding proteins on the endoplasmic reticulum and focal adhesions, perhaps by regulation of tyrosine phosphorylation, inhibits signal transduction. Tyrosine phosphorylation has important implications for the dysfunctional tissue and organ fibrosis observed in such devastating diseases as pulmonary fibrosis, chronic obstructive lung disease, and rheumatoid arthritis.

Opportunities for drug development

As PTPs are known to modulate pivotal signalling pathways involved in immune, inflammatory and fibrotic responses, selective modulation of these pathways by strategies that target PTPs is an appealing concept. There are certainly precedents for targeting tyrosine kinases

with molecular therapeutics. Recent studies have demonstrated the effectiveness of tyrosine kinase inhibitors in the treatment of a variety of cancers. For example, imatinib mesylate (Gleevec; Novartis), an oral tyrosine kinase inhibitor that targets BCR–Abl, c-Kit and PDGF receptors- α and - β (both tyrosine kinases), has been shown to be effective in the treatment of chronic myelogenous leukaemia and a variety of other cancers^{109,110}. Strategies that target the EGF receptor with small-molecule tyrosine kinase inhibitors such as gefitinib (Iressa; AstraZeneca)¹¹¹ and EKB-569 (Wyeth-Ayerst)¹¹², or which use blocking monoclonal antibodies such as matuzumab (EMD Pharmaceuticals)¹¹³, have also shown promise in the treatment of several cancers. The small-molecule tyrosine kinase inhibitor SU5416 (semaxanib; Sugen), which targets the vascular endothelial growth factor receptor, has also shown promise as an antineoplastic treatment^{114,115}. Additionally, agents that target tyrosine kinases such as JAK family members are being developed as immunomodulatory agents for the treatment of immune and inflammatory disorders¹¹⁶.

Compared with the therapeutic use of tyrosine kinase inhibitors, much less information is available in the public domain on therapeutic approaches that target PTPs. Nonetheless, the discovery of small molecules that regulate PTPs is a subject of active investigation by both academic and pharmaceutical researchers¹¹⁷. On first consideration, targeting PTPs poses several challenges. First, the catalytic domains ('signature motif') of all classical P-tyr-directed PTPs are highly conserved. Therefore non-selective inhibitors directed against the phosphatase domain might affect many (or all) of the 100+ distinct PTPs encoded by the human genome. Many of these PTPs regulate key signalling pathways involved in numerous essential cellular processes^{118–120}, therefore increasing the likelihood of unintended and untoward ('off target') effects. Surprisingly, despite this potential shortcoming, the broad-range PTP inhibitor vanadate and its derivatives have shown promise in the treatment of diabetes mellitus in both animal models and humans^{121–129}. Second, PTPs act as negative regulators in many (but not all) signalling pathways, including those triggered by growth factor receptors. For these pathways, activation as opposed to inhibition of selective PTPs might be a more appropriate strategy. Third, individual PTPs might, like tyrosine kinases, function in multiple pathways, again increasing the probability of off-target effects. Fourth, some potent PTP inhibitors contain a functionality that mimics a phosphate group (and therefore contains a negative charge), which reduces their capacity to permeate membranes and creates problems in achieving efficient intestinal absorption¹¹⁷. Possible solutions to these issues include the use of enzymatically cleavable moieties to mask the charged domains, or using carrier molecules to facilitate absorption in the gastrointestinal tract.

On the other hand, PTPs have several distinctive features that provide potential targets for therapeutic manipulation. Despite the highly conserved nature of the core catalytic domain, the surrounding parts of the substrate-binding 'groove' of many PTPs are sufficiently

structurally distinct to allow selective binding of substrates and therefore selective modulation by small-molecule inhibitors or activators¹³⁰. Furthermore, many PTPs are receptor-like and as such present extracellular domains that are potential targets of molecules such as peptides or antibodies. Importantly, as discussed above, the non-catalytic domains of PTPs are structurally distinct and are known to regulate the enzymatic activity of the phosphatase (catalytic) domain and to mediate highly specific protein–protein interactions that are crucial for regulating cellular signalling pathways. Examples include the SH2 domains of SHP1^{131,132} and SHP2^{85,133,134}, and the lipid binding domain of MEG2 which has homology to the cellular retinaldehyde-binding protein¹³⁵, which modulate the catalytic activity of PTPs, target PTPs to specific subcellular domains, and serve to recruit specific binding partners to multimeric signalling complexes¹³⁵. Small molecules that bind specifically to these distinctive domains and modulate the catalytic activity and/or adaptor functions of PTPs represent viable approaches as therapeutic agents. Such strategies hold great promise for the treatment of diverse diseases that impose a huge burden on society, including diabetes mellitus, obesity, and immune, inflammatory and fibrotic disorders^{108,130,136}.

Selective modulation of signalling pathways triggered by IL-1, especially those that are focal adhesion-dependent, is a potential therapeutic strategy for the amelioration of inflammatory tissue injury. As this represents relatively uncharted territory, researchers would do well to draw on the experience obtained with developing PTP inhibitors for other diseases, such as diabetes, to expedite the development of PTP inhibitors for the treatment of inflammatory disorders. In this regard, a small-molecule inhibitor of SHP2, NCS-87877, has recently been described that binds to the catalytic cleft of SHP2, thereby inhibiting its phosphatase activity¹³⁷. This compound selectively inhibited EGF-induced ERK activation in cultured cells without affecting ERK activation by other stimuli, raising the possibility of its use in modulating SHP2-dependent signalling pathways that mediate inflammatory tissue injury. Clearly, such an approach must be undertaken judiciously because PTPs such as SHP2 and PTP α participate in signalling pathways that regulate physiologically important processes in structural and immune cells. However, the local delivery of therapeutic agents for brief periods of time into an inflammatory milieu such as the joint or the lung might allow some selectivity in the modulation of signalling pathways.

Conclusions

Therapeutic control of inflammation is essential for the clinical management of a wide range of high-prevalence human diseases including asthma, pulmonary fibrosis, rheumatoid arthritis, osteoarthritis, periodontitis, Crohn's disease, atheroma formation, multiple sclerosis and an expanding group of auto-inflammatory disorders. Inhibition of TNF α is effective in most patients with resistant rheumatoid arthritis. IL-1 inhibition seems to be more effective in controlling the manifestations of several auto-inflammatory syndromes. However, as

available agents have important shortcomings, including cost and the lack of predictability in clinical response (both during initiation and maintenance), there is a clear need to develop alternative approaches for inhibiting TNF α and IL-1 in a more predictable and cost-effective manner. The development of small molecules and peptides to target the intracellular signalling pathways of these cytokines, including novel anti-inflammatory drug targets based on the clustering of IL-1 receptors into large, multi-protein aggregates, is a promising avenue to pursue. Interference with receptor aggregation and the functional relationships between receptors and the endoplasmic reticulum effectively abrogates IL-1 signalling. In chondrocytes and fibroblasts in particular,

the IL-1-generated signal relies on the clustering of receptors into cell adhesion complexes, which can be dissipated by low-molecular-mass peptides that block IL-1-generated, pro-inflammatory signals. The assembly of the adhesion complex and its functional connection to signal generation from the endoplasmic reticulum seems to be highly dependent on protein tyrosine phosphatases, which are abundant in adhesion complexes and mutation of which is associated with poorly controlled inflammatory disease. We suggest that protein tyrosine phosphatases, particularly SHP2, could provide a particularly rich array of targets for the development of low-molecular-mass peptides for anti-inflammatory drug development.

1. Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448–454 (1999).
2. Singh, R. *et al.* The IL-1 receptor and Rho directly associate to drive cell activation in inflammation. *J. Clin. Invest.* **103**, 1561–1570 (1999).
3. Lipsky, P. E. *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med.* **343**, 1594–1602 (2000).
4. Genovese, M. C. *et al.* Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes. *Arthritis Rheum.* **46**, 1443–1450 (2002).
5. Bathon, J. M. *et al.* A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N. Engl. J. Med.* **343**, 1586–1593 (2000).
6. Breedveld, F. C. *et al.* The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum.* **54**, 26–37 (2006).
7. Keystone, E. C. *et al.* Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum.* **50**, 1400–1411 (2004).
8. Smolen, J. S. *et al.* Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. *Arthritis Rheum.* **52**, 1020–1030 (2005).
9. Deng, G. M., Zheng, L., Chan, F. K. & Lenardo, M. Amelioration of inflammatory arthritis by targeting the pre-ligand assembly domain of tumor necrosis factor receptors. *Nature Med.* **11**, 1066–1072 (2005).
10. Horai, R. *et al.* TNF- α is crucial for the development of autoimmune arthritis in IL-1 receptor antagonist-deficient mice. *J. Clin. Invest.* **114**, 1603–1611 (2004).
11. Redlich, K. *et al.* Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. *Am. J. Pathol.* **164**, 543–555 (2004).
12. Zwerina, J. *et al.* Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. *Arthritis Rheum.* **50**, 277–290 (2004).
13. Redlich, K. *et al.* Osteoclasts are essential for TNF- α -mediated joint destruction. *J. Clin. Invest.* **110**, 1419–1427 (2002).
14. Shealy, D. J. *et al.* Anti-TNF- α antibody allows healing of joint damage in polyarthritic transgenic mice. *Arthritis Res.* **4**, R7 (2002).
15. Keystone, E. C. & Kavanaugh, A. What to do with TNF failures. *Expert Opin. Drug Saf.* **4**, 149–155 (2005).
16. Smith, D. E. *et al.* Four new members expand the interleukin-1 superfamily. *J. Biol. Chem.* **275**, 1169–1175 (2000).
17. O'Neill, L. A. & Dinarello, C. A. The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense. *Immunol. Today* **21**, 206–209 (2000).
18. Goldring, S. R. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. *Rheumatology (Oxford)* **42** Suppl 2, ii11–116 (2003).
19. Dayer, J. M. & Bresnihan, B. Targeting interleukin-1 in the treatment of rheumatoid arthritis. *Arthritis Rheum.* **46**, 574–578 (2002).
20. Jindal, S. K. & Agarwal, R. Autoimmunity and interstitial lung disease. *Curr. Opin. Pulm. Med.* **11**, 438–446 (2005).
21. Loos, B. G., John, R. P. & Laine, M. L. Identification of genetic risk factors for periodontitis and possible mechanisms of action. *J. Clin. Periodontol.* **32** (Suppl. 6), 159–179 (2005).
22. Bresnihan, B. & Cunnane, C. Interleukin-1 receptor antagonist. *Rheum. Dis. Clin. North Am.* **24**, 615–628 (1998).
23. Braddock, M. & Quinn, A. Targeting IL-1 in inflammatory disease: new opportunities for therapeutic intervention. *Nature Rev. Drug Discov.* **3**, 330–339 (2004).
24. Christodoulou, C. & Choy, E. H. Joint inflammation and cytokine inhibition in rheumatoid arthritis. *Clin. Exp. Med.* **6**, 13–19 (2006).
25. Janssens, S. & Beyaert, R. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol. Cell* **11**, 293–302 (2003).
26. Auron, P. E. The interleukin 1 receptor: ligand interactions and signal transduction. *Cytokine Growth Factor Rev.* **9**, 221–237 (1998).
27. Dower, S. K. *et al.* Detection and characterization of high affinity plasma membrane receptors for human interleukin 1. *J. Exp. Med.* **162**, 501–515 (1985).
28. Akira, S., Takeda, K. & Kaisho, T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nature Immunol.* **2**, 675–680 (2001).
29. Sims, J. E. *et al.* Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc. Natl. Acad. Sci. USA* **90**, 6155–6159 (1993).
30. Wesche, H. *et al.* The interleukin 1 β receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). *J. Biol. Chem.* **272**, 7727–7731 (1997).
31. Burns, K. *et al.* Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nature Cell Biol.* **2**, 346–351 (2000).
32. Burns, K. *et al.* MyD88, an adapter protein involved in interleukin-1 signaling. *J. Biol. Chem.* **273**, 12203–12209 (1998).
33. Qvarnstrom, E. E., MacFarlane, S. A., Page, R. C. & Dower, S. K. Interleukin 1 β induces rapid phosphorylation and redistribution of talin: a possible mechanism for modulation of fibroblast focal adhesion. *Proc. Natl. Acad. Sci. USA* **88**, 1232–1236 (1991).
34. Palsson, E. M., Popoff, M., Thelestam, M. & O'Neill, L. A. Divergent roles for Ras and Rap in the activation of p38 mitogen-activated protein kinase by interleukin-1. *J. Biol. Chem.* **275**, 7818–7825 (2000).
35. Matthews, J. S. & O'Neill, L. A. Distinct roles for p42/p44 and p38 mitogen-activated protein kinases in the induction of IL-2 by IL-1. *Cytokine* **11**, 643–655 (1999).
36. Luo, L., Cruz, T. & McCulloch, C. Interleukin 1-induced calcium signalling in chondrocytes requires focal adhesions. *Biochem. J.* **324**, 653–658 (1997).
37. Zhu, P., Xiong, W., Rodgers, G. & Qvarnstrom, E. E. Regulation of interleukin 1 signalling through integrin binding and actin reorganization: disparate effects on NF- κ B and stress kinase pathways. *Biochem. J.* **330**, 975–981 (1998).
38. Maraldi, N. M., Marmiroli, S., Rizzoli, R., Mazzotti, G. & Manzoli, F. A. Phosphatidylinositol 3-kinase translocation to the nucleus is an early event in the interleukin-1 signalling mechanism in human osteosarcoma Saos-2 cells. *Adv. Enzyme Regul.* **39**, 33–49 (1999).
39. Bergman, M. R. *et al.* A functional activating protein 1 (AP-1) site regulates matrix metalloproteinase 2 (MMP-2) transcription by cardiac cells through interactions with JunB-Fra1 and JunB-FosB heterodimers. *Biochem. J.* **369**, 485–496 (2003).
40. Ferrari, D. *et al.* The P2X7 receptor: a key player in IL-1 processing and release. *J. Immunol.* **176**, 3877–3883 (2006).
41. Laliberte, R. E. *et al.* Glutathione s-transferase omega 1-1 is a target of cytokine release inhibitory drugs and may be responsible for their effect on interleukin-1 β posttranslational processing. *J. Biol. Chem.* **278**, 16567–16578 (2003).
42. Smeets, R. L. *et al.* Soluble interleukin-1 receptor accessory protein ameliorates collagen-induced arthritis by a different mode of action from that of interleukin-1 receptor antagonist. *Arthritis Rheum.* **52**, 2202–2211 (2005).
43. Fleischmann, R. M. *et al.* Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: A large, international, multicenter, placebo-controlled trial. *Arthritis Rheum.* **48**, 927–934 (2003).
44. Siegel, J. FDA briefing document on safety and efficacy update of approved TNF-blocking agents [online], <http://www.fda.gov/OHRMS/DOCKETS/ac/03/slides/3930s1.htm> (2003).
45. Zou, J. *et al.* Down-regulation of the nonspecific and antigen-specific T cell cytokine response in ankylosing spondylitis during treatment with infliximab. *Arthritis Rheum.* **48**, 780–790 (2003).
46. Bresnihan, B., Newmark, R., Robbins, S. & Genant, H. K. Effects of anakinra monotherapy on joint damage in patients with rheumatoid arthritis. Extension of a 24-week randomized, placebo-controlled trial. *J. Rheumatol.* **31**, 1103–1111 (2004).
47. Cohen, S. *et al.* Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* **46**, 614–624 (2002).

48. Jiang, Y. *et al.* A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum.* **43**, 1001–1009 (2000).
49. Coxon, A. *et al.* Inhibition of interleukin-1 but not tumor necrosis factor suppresses neovascularization in rat models of corneal angiogenesis and adjuvant arthritis. *Arthritis Rheum.* **46**, 2604–2612 (2002).
50. Bendele, A. *et al.* Efficacy of sustained blood levels of interleukin-1 receptor antagonist in animal models of arthritis: comparison of efficacy in animal models with human clinical data. *Arthritis Rheum.* **42**, 498–506 (1999).
51. Boivin, B., Villeneuve, L. R., Farhat, N., Chevalier, D. & Allen, B. G. Sub-cellular distribution of endothelin signaling pathway components in ventricular myocytes and heart: lack of preformed caveolar signalosomes. *J. Mol. Cell. Cardiol.* **38**, 665–676 (2005).
52. Pascual, V., Allantaz, F., Arce, E., Punaro, M. & Bancchereau, J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J. Exp. Med.* **201**, 1479–1486 (2005).
53. Fitzgerald, A. A., Leclercq, S. A., Yan, A., Homik, J. E. & Dinarello, C. A. Rapid responses to anakinra in patients with refractory adult-onset Still's disease. *Arthritis Rheum.* **52**, 1794–1803 (2005).
54. Stojanov, S. & Kastner, D. L. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Curr. Opin. Rheumatol.* **17**, 586–599 (2005).
55. Drenth, J. P. & van der Meer, J. W. The Inflammasome—A Linebacker of Innate Defense. *N. Engl. J. Med.* **355**, 730–732 (2006).
56. Srinivasula, S. M. *et al.* The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. *J. Biol. Chem.* **277**, 21119–21122 (2002).
57. Lovell, D. J., Bowyer, S. L. & Solinger, A. M. Interleukin-1 blockade by anakinra improves clinical symptoms in patients with neonatal-onset multisystem inflammatory disease. *Arthritis Rheum.* **52**, 1283–1286 (2005).
58. Boschan, C. *et al.* Neonatal-onset multisystem inflammatory disease (NOMID) due to a novel S331R mutation of the CIAS1 gene and response to interleukin-1 receptor antagonist treatment. *Am. J. Med. Genet. A* **140**, 883–886 (2006).
59. Rudolph, K., Gerwin, N., Verzijl, N., van der Kraan, P. & van den Berg, W. Pralinasan, an inhibitor of interleukin-1 β converting enzyme, reduces joint damage in two murine models of osteoarthritis. *Osteoarthritis Cartilage* **11**, 738–746 (2003).
60. Loher, F. *et al.* The interleukin-1 β -converting enzyme inhibitor pralinasan reduces dextran sulfate sodium-induced murine colitis and T helper 1 T-cell activation. *J. Pharmacol. Exp. Ther.* **308**, 583–590 (2004).
61. Pavelka, K. Clinical effects of pralinasan (PRAL), an orally-active interleukin-1 β converting enzyme (ICE) inhibitor in a 285-patient phase II trial in rheumatoid arthritis (RA). *Arthritis Rheum.* **46** (Suppl. 9), S281 (2002).
62. Economides, A. N. *et al.* Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nature Med.* **9**, 47–52 (2003).
63. Guler, H.-P., Caldwell, J., Littlejohn, Tl, Mcllwain, H., Offenberg, H., Stahl, N. . A phase I, single dose escalation study of IL-1 trap in patients with rheumatoid arthritis. *Arthritis Rheum.* **44**, S370 (2001).
64. Breiting, R. & Hoeller, D. Current challenges in quantitative modeling of epidermal growth factor signaling. *FEBS Lett.* **579**, 6289–6294 (2005).
65. Richardson, K. S. & Zundel, W. The emerging role of the COP9 signalosome in cancer. *Mol. Cancer Res.* **3**, 645–653 (2005).
66. Noguchi, T. *et al.* Recruitment of tumor necrosis factor receptor-associated factor family proteins to apoptosis signal-regulating kinase 1 signalosome is essential for oxidative stress-induced cell death. *J. Biol. Chem.* **280**, 37033–37040 (2005).
67. Kapoor, G. S., Zhan, Y., Johnson, G. R. & O'Rourke, D. M. Distinct domains in the SHP-2 phosphatase differentially regulate epidermal growth factor receptor/NF- κ B activation through Gab1 in glioblastoma cells. *Mol. Cell Biol.* **24**, 823–836 (2004).
68. Arora, P. D., Ma, J., Min, W., Cruz, T. & McCulloch, C. A. Interleukin-1-induced calcium flux in human fibroblasts is mediated through focal adhesions. *J. Biol. Chem.* **270**, 6042–6049 (1995).
69. Qwarnstrom, E. E., Page, R. C., Gillis, S. & Dower, S. K. Binding, internalization, and intracellular localization of interleukin-1 β in human diploid fibroblasts. *J. Biol. Chem.* **263**, 8261–8269 (1988).
70. Critchley, D. R. Focal adhesions — the cytoskeletal connection. *Curr. Opin. Cell Biol.* **12**, 133–139 (2000).
71. Burridge, K. & Chrzanoska-Wodnicka, M. Focal adhesions, contractility, and signaling. *Annu. Rev. Cell Dev. Biol.* **12**, 463–518 (1996).
72. Wehrle-Haller, B. & Imhof, B. The inner lives of focal adhesions. *Trends Cell Biol.* **12**, 382–389 (2002).
73. Carragher, N. O. & Frame, M. C. Focal adhesion and actin dynamics: a place where kinases and proteases meet to promote invasion. *Trends Cell Biol.* **14**, 241–249 (2004).
74. Zamir, E. & Geiger, B. Molecular complexity and dynamics of cell-matrix adhesions. *J. Cell Sci.* **114**, 3583–3590 (2001).
75. MacGillivray, M. K., Cruz, T. F. & McCulloch, C. A. The recruitment of the interleukin-1 (IL-1) receptor-associated kinase (IRAK) into focal adhesion complexes is required for IL-1 β -induced ERK activation. *J. Biol. Chem.* **275**, 23509–23515 (2000).
76. Murphy-Ullrich, J. E., Gurusiddappa, S., Frazier, W. A. & Hook, M. Heparin-binding peptides from thrombospondins 1 and 2 contain focal adhesion-labilizing activity. *J. Biol. Chem.* **268**, 26784–26789 (1993).
77. Wang, Q., Downey, G. P., Choi, C., Kapus, A. & McCulloch, C. A. IL-1 induced release of Ca²⁺ from internal stores is dependent on cell-matrix interactions and regulates ERK activation. *FASEB J.* **17**, 1898–1900 (2003).
78. Zaidel-Bar, R., Cohen, M., Addadi, L. & Geiger, B. Hierarchical assembly of cell-matrix adhesion complexes. *Biochem. Soc. Trans.* **32**, 416–420 (2004).
79. Kirchner, J., Kam, Z., Tzur, G., Bershadsky, A. D. & Geiger, B. Live-cell monitoring of tyrosine phosphorylation in focal adhesions following microtubule disruption. *J. Cell Sci.* **116**, 975–986 (2003).
80. Ilic, D. *et al.* Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. *Nature* **377**, 539–544 (1995).
81. Shinkai, Y. *et al.* RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867 (1992).
82. Klinghoffer, R. A., Sachsenmaier, C., Cooper, J. A. & Soriano, P. Src family kinases are required for integrin but not PDGFR signal transduction. *EMBO J.* **18**, 2459–2471 (1999).
83. Miyamoto, S., Akiyama, S. K. & Yamada, K. M. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* **267**, 883–885 (1995).
84. Katz, B. Z. *et al.* Targeting membrane-localized focal adhesion kinase to focal adhesions: roles of tyrosine phosphorylation and SRC family kinases. *J. Biol. Chem.* **278**, 29115–29120 (2003).
85. Herrera Abreu, M. T. *et al.* Tyrosine phosphatase SHP-2 regulates IL-1 signaling in fibroblasts through focal adhesions. *J. Cell Physiol.* **207**, 132–143 (2006).
86. Alonso, A. *et al.* Protein tyrosine phosphatases in the human genome. *Cell* **117**, 699–711 (2004).
87. Ayalon, O. & Geiger, B. Cyclic changes in the organization of cell adhesions and the associated cytoskeleton, induced by stimulation of tyrosine phosphorylation in bovine aortic endothelial cells. *J. Cell Sci.* **110**, 547–556 (1997).
88. Jones, M. L. & Poole, A. W. Protein tyrosine phosphatases. *Methods Mol. Biol.* **273**, 169–78 (2004).
89. Tonks, N. K. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* **121**, 667–670 (2005).
90. Poole, A. W. & Jones, M. L. A SHPping tale: perspectives on the regulation of SHP-1 and SHP-2 tyrosine phosphatases by the C-terminal tail. *Cell Signal* **17**, 1323–1332 (2005).
91. Chishti, A. H. *et al.* The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends Biochem. Sci.* **23**, 281–282 (1998).
92. Fujioka, Y. *et al.* A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. *Mol. Cell Biol.* **16**, 6887–6899 (1996).
93. Tsuda, M. *et al.* Integrin-mediated tyrosine phosphorylation of SHPS-1 and its association with SHP-2. Roles of Fak and Src family kinases. *J. Biol. Chem.* **273**, 13223–13229 (1998).
94. Yu, D. H., Qu, C. K., Henegariu, O., Lu, X. & Feng, G. S. Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. *J. Biol. Chem.* **273**, 21125–21131 (1998).
95. Oh, E. S. *et al.* Regulation of early events in integrin signaling by protein tyrosine phosphatase SHP-2. *Mol. Cell Biol.* **19**, 3205–3215 (1999).
96. Kodama, A. *et al.* Involvement of a SHP-2-Rho small G protein pathway in hepatocyte growth factor/scatter factor-induced cell scattering. *Mol. Biol. Cell* **11**, 2565–2575 (2000).
97. Schoenwaelder, S. M. *et al.* The protein tyrosine phosphatase Shp-2 regulates RhoA activity. *Curr. Biol.* **10**, 1523–1526 (2000).
98. Kodama, A. *et al.* Regulation of Ras and Rho small G proteins by SHP-2. *Genes Cells* **6**, 869–876 (2001).
99. MacGillivray, M. *et al.* The protein tyrosine phosphatase SHP-2 regulates interleukin-1-induced ERK activation in fibroblasts. *J. Biol. Chem.* **278**, 27190–27198 (2003).
100. Wang, Q., Downey, G. P., Herrera-Abreu, M. T., Kapus, A. & McCulloch, C. A. SHP-2 modulates interleukin-1-induced Ca²⁺ flux and ERK activation via phosphorylation of phospholipase C γ 1. *J. Biol. Chem.* **280**, 8397–8406 (2005).
101. Lo, Y. Y., Luo, L., McCulloch, C. A. & Cruz, T. F. Requirements of focal adhesions and calcium fluxes for interleukin-1-induced ERK kinase activation and c-fos expression in fibroblasts. *J. Biol. Chem.* **273**, 7059–7065 (1998).
102. Lammers, R., Lerch, M. M. & Ullrich, A. The carboxyl-terminal tyrosine residue of protein-tyrosine phosphatase α mediates association with focal adhesion plaques. *J. Biol. Chem.* **275**, 3391–3396 (2000).
103. von Wichert, G. *et al.* RPTP- α acts as a transducer of mechanical force on α v β 3-integrin-cytoskeleton linkages. *J. Cell Biol.* **161**, 143–153 (2003).
104. Su, J., Muranjan, M. & Sap, J. Receptor protein tyrosine phosphatase α activates Src-family kinases and controls integrin-mediated responses in fibroblasts. *Curr. Biol.* **9**, 505–511 (1999).
105. Hardingham, G. E., Cruzalegui, F. H., Chawla, S. & Bading, H. Mechanisms controlling gene expression by nuclear calcium signals. *Cell Calcium* **23**, 131–134 (1998).
106. Ma, H. T. *et al.* Requirement of the inositol trisphosphate receptor for activation of store-operated Ca²⁺ channels. *Science* **287**, 1647–1651 (2000).
107. Patterson, R. L., Boehning, D. & Snyder, S. H. Inositol 1, 4, 5-trisphosphate receptors as signal integrators. *Annu. Rev. Biochem.* **73**, 437–465 (2004).
108. Kruger, J. *et al.* Deficiency of Src homology 2-containing phosphatase 1 results in abnormalities in murine neutrophil function: studies in motheaten mice. *J. Immunol.* **165**, 5847–5859 (2000).
109. Marucci, G., Perrotti, D. & Caligiuri, M. A. Understanding the molecular basis of imatinib mesylate therapy in chronic myelogenous leukemia and the related mechanisms of resistance. *Clin. Cancer Res.* **9**, 1333–1337 (2003).
110. Ross, D. M. & Hughes, T. P. Cancer treatment with kinase inhibitors: what have we learnt from imatinib? *Br. J. Cancer* **90**, 12–19 (2004).
111. West, H. L. *et al.* Gefitinib therapy in advanced bronchioloalveolar carcinoma: Southwest Oncology Group Study S0126. *J. Clin. Oncol.* **24**, 1807–1813 (2006).
112. Erlichman, C. *et al.* Phase I study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor, in patients with advanced solid tumors. *J. Clin. Oncol.* **24**, 2252–2260 (2006).
113. Graeven, U. *et al.* Phase I study of the humanised anti-EGFR monoclonal antibody matuzumab (EMD 72000) combined with gemcitabine in advanced pancreatic cancer. *Br. J. Cancer* **94**, 1293–1299 (2006).
114. Jennens, R. R., Rosenthal, M. A., Lindeman, G. J. & Michael, M. Complete radiological and metabolic response of metastatic renal cell carcinoma to SU5416 (semaxanib) in a patient with probable von Hippel-Lindau syndrome. *Urol. Oncol.* **22**, 195–196 (2004).
115. Yoshinari, N. *et al.* Effects of scaling and root planing on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1 β in gingival crevicular fluid and gingival tissues. *J. Periodontol. Res.* **39**, 158–167 (2004).

116. Papageorgiou, A. C. & Wikman, L. E. Is JAK3 a new drug target for immunomodulation-based therapies? *Trends Pharmacol. Sci.* **25**, 558–562 (2004).
117. Johnson, T. O., Ermolieff, J. & Jirousek, M. R. Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nature Rev. Drug Discov.* **1**, 696–709 (2002).
118. Alonso, A. *et al.* Protein tyrosine phosphatases in the human genome. *Cell* **117**, 699–711 (2004).
119. Mustelin, T., Vang, T. & Bottini, N. Protein tyrosine phosphatases and the immune response. *Nature Rev. Immunol.* **5**, 43–57 (2005).
120. Tautz, L., Pellicchia, M. & Mustelin, T. Targeting the PTome in human disease. *Expert Opin Ther Targets* **10**, 157–177 (2006).
121. Pederson, R. A., Ramanadham, S., Buchan, A. M. & McNeill, J. H. Long-term effects of vanadyl treatment on streptozocin-induced diabetes in rats. *Diabetes* **38**, 1390–1395 (1989).
122. Meyerovitch, J., Rothenberg, P., Shechter, Y., Bonner-Weir, S. & Kahn, C. R. Vanadate normalizes hyperglycemia in two mouse models of non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **87**, 1286–1294 (1991).
123. Robertson, R. P. & Klein, D. J. Treatment of diabetes mellitus. *Diabetologia* **35** (Suppl. 2), S8–S17 (1992).
124. Goldfine, A. B., Simonson, D. C., Folli, F., Patti, M. E. & Kahn, C. R. Metabolic effects of sodium metavanadate in humans with insulin-dependent and noninsulin-dependent diabetes mellitus *in vivo* and *in vitro* studies. *J. Clin. Endocrinol. Metab.* **80**, 3311–3320 (1995).
125. Sakurai, H. A new concept: the use of vanadium complexes in the treatment of diabetes mellitus. *Chem. Rec.* **2**, 237–248 (2002).
126. Winter, C. L. *et al.* A nonspecific phosphotyrosine phosphatase inhibitor, bis(maltolato)oxovanadium(IV), improves glucose tolerance and prevents diabetes in Zucker diabetic fatty rats. *Exp. Biol. Med. (Maywood)* **230**, 207–216 (2005).
127. Poucheret, P., Verma, S., Grynepas, M. D. & McNeill, J. H. Vanadium and diabetes. *Mol. Cell. Biochem.* **188**, 73–80 (1998).
128. Sekar, N., Li, J. & Shechter, Y. Vanadium salts as insulin substitutes: mechanisms of action, a scientific and therapeutic tool in diabetes mellitus research. *Crit. Rev. Biochem. Mol. Biol.* **31**, 339–359 (1996).
129. Willsky, G. R. *et al.* Effect of vanadium(IV) compounds in the treatment of diabetes: *in vivo* and *in vitro* studies with vanadyl sulfate and bis(maltolato)oxovanadium(IV). *J. Inorg. Biochem.* **85**, 33–42 (2001).
130. Rao, G. S., Ramachandran, M. V. & Bajaj, J. S. *In silico* structure-based design of a potent and selective small peptide inhibitor of protein tyrosine phosphatase 1B, a novel therapeutic target for obesity and type 2 diabetes mellitus: a computer modeling approach. *J. Biomol. Struct. Dyn.* **23**, 377–384 (2006).
131. Mizuno, K., Katagiri, T., Hasegawa, K., Ogimoto, M. & Yakura, H. Hematopoietic cell phosphatase, SHP-1, is constitutively associated with the SH2 domain-containing leukocyte protein, SLP-76, in B cells. *J. Exp. Med.* **184**, 457–463 (1996).
132. Jiao, H. *et al.* Direct association with and dephosphorylation of Jak2 kinase by the SH2-domain-containing protein tyrosine phosphatase SHP-1. *Mol. Cell Biol.* **16**, 6985–6992 (1996).
133. Barford, D. & Neel, B. G. Revealing mechanisms for SH2 domain mediated regulation of the protein tyrosine phosphatase SHP-2. *Structure* **6**, 249–254 (1998).
134. Huyer, G. & Ramachandran, C. The specificity of the N-terminal SH2 domain of SHP-2 is modified by a single point mutation. *Biochemistry* **37**, 2741–2747 (1998).
135. Kruger, J. M. *et al.* Protein-tyrosine phosphatase MEG2 is expressed by human neutrophils. Localization to the phagosome and activation by polyphosphoinositides. *J. Biol. Chem.* **277**, 2620–2628 (2002).
136. Zhang, Z. Y. & Lee, S. Y. PTP1B inhibitors as potential therapeutics in the treatment of type 2 diabetes and obesity. *Expert Opin. Investig. Drugs* **12**, 223–233 (2003).
137. Chen, L. *et al.* Discovery of a novel shp2 protein tyrosine phosphatase inhibitor. *Mol. Pharmacol.* **70**, 562–570 (2006).
138. Zhang, S. Q., Kovalenko, A., Cantarella, G. & Wallach, D. Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKK γ) upon receptor stimulation. *Immunity* **12**, 301–311 (2000).

Acknowledgements

C.A.M., G.P.D and H.E.G. are supported by operating grants from the Canadian Institutes of Health Research.

Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

Caspase 1 | c-FOS | EGF | Glutathione S-transferase omega 1-1 | IL-1 | IL-1RI | IL-1RII | IL-1RA | IL-1RacP | IRAK1 | IRAK2 | MYD88 | SHP2 | TOLLIP | TRAF6

OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>

Ankylosing spondylitis | Atherosclerosis | Inflammatory bowel disease | Psoriatic arthritis | Pulmonary fibrosis | Rheumatoid arthritis | Systemic lupus erythematosus

FURTHER INFORMATION

CIHR Group in Matrix Dynamics:

<http://www.cihrmatrix.ca/>

Access to this interactive links box is free online.