Drug targets in inflammation and immunomodulation

Derek R. Buckle and Charles J.R. Hedgecock

Major advances in our understanding of inflammation and immunomodulation at both the biological and molecular level have opened up multiple opportunities for therapeutic intervention. Some of the more established of these approaches have now been validated clinically, but there is still a long way to go before the mainstay treatments such as steroids and the nonsteroidal anti-inflammatory drugs have significant competition. This review attempts to summarize some of the ongoing activities in this area, while recognizing that a truly comprehensive insight into many exciting research activities is not possible.

As the host response to infection or tissue destruction, inflammatory cell recruitment is an essential protective and repair process that is subject to control by diverse modulatory mechanisms. Under ideal conditions, inflammatory events occur with a minimum of vascular and local tissue disruption; excessive responses lead to significant clinical manifestations that may necessitate therapeutic intervention. Classical agents, such as the corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), are the mainstay treatments for inflammatory disorders, but both induce adverse side effects resulting from inadequate selectivity of action. A diverse array of new approaches is currently under active investigation within the pharmaceutical industry, many having resulted in potent and selective therapeutic agents that have been evaluated clinically. Other targets have yet to produce useful chemical entities, or still await conclusive evidence to support their therapeutic utility. This review focuses on some of the more important approaches that have been studied, and attempts to summarize what is currently known.

Inhibitors of arachidonic acid release and metabolism

The release of arachidonic acid (eicosatetra-5,8,11,14-enoic acid) from its phospholipid stores in response to a variety of stimuli leads to a complex plethora of metabolites, many of which elicit significant inflammatory properties[1,2]. Two major pathways of arachidonic acid metabolism are known: that mediated by cyclo-oxygenase, leading to prostaglandins, and that mediated by 5-lipoxygenase, leading to leukotrienes and other lipoxygenase products (Figure 1). Intervention at various points within the arachidonic acid cascade has long been seen as an attractive therapeutic option and a wide spectrum of inhibitors or selective antagonists is now available[3].

Cyclo-oxygenase inhibitors

NSAIDs are well-established inhibitors of cyclo-oxygenase, but have the particular disadvantage of gastrointestinal toxicity, caused by the inhibition of cytoprotective prostaglandins in the stomach and gut. The discovery of an inducible cyclo-oxygenase, COX-2, which is markedly upregulated at inflammatory sites, has opened possibilities for finding selective inhibitors with reduced gastrointestinal side effects[4]. It is remarkable that while the active site of

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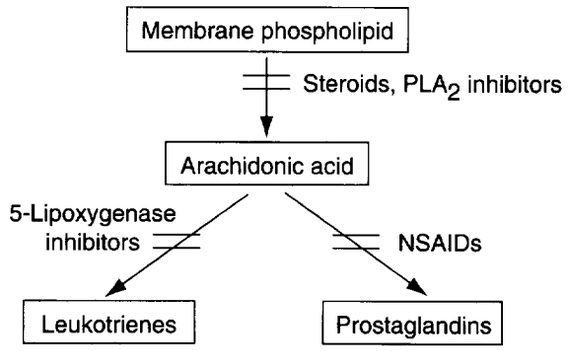


Figure 1. *Prostaglandin and leukotriene pathways.*

COX-2 differs from that of the more ubiquitous enzyme, COX-1, by only a single amino acid, selective inhibitors are nevertheless known. Typical among these inhibitors are celecoxib (SC58635) and MK966 (L745337) (Figure 2), which show marked selectivity for the inducible enzyme and are active in animal models of inflammatory disease at doses that do not cause gastric ulceration[4,5]. Until the results of ongoing clinical trials are reported it will not be known whether these more selective COX-2 inhibitors show therapeutic utility in the absence of gastric toxicity, although early reports with celecoxib in rheumatoid arthritis and osteoarthritis patients are encouraging[6].

Leukotriene modulators

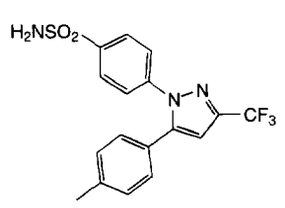
Leukotrienes (LT) fall into two distinct classes, one contains amino acid moieties (peptidoleukotrienes: LTC_4, LTD_4 and LTE_4), and the other a dihydroxy-eicosanoid, LTB_4 (Figure 2). Both classes of leukotriene are products of the conversion of arachidonic acid by 5-lipoxygenase, and both are strongly implicated in inflammation and allergic disease[7], leading to considerable focus on inhibitors of leukotriene formation as well as specific receptor antagonists. Because lipoxygenation is an oxidative process, various antioxidants show lipoxygenase inhibitory activity, and many compounds identified as inhibitors of 5-lipoxygenase are also antioxidants[8]. Whether such activity is a disadvantage is not known, but the recently launched 5-lipoxygenase inhibitor zileuton (Figure 2), designed to interact with the catalytic iron ion of 5-lipoxygenase, shares this activity and appears to be well tolerated in man[9,10]. Zileuton is proposed as an alternative to low-dose inhaled steroids, cromoglycate or nedocromil for the treatment of asthma.

Inhibitors of the protein responsible for the activation of 5-lipoxygenase (5-lipoxygenase activating protein, FLAP) offer an alternative approach to the inhibition of 5-lipoxygenase, and a number of quinolines, epitomized by MK0591 (Figure 2), have been identified for further development[3,11]. While inhibitors of FLAP are less likely to share the antioxidant activity common to many 5-lipoxygenase inhibitors, and may well have a better long-term profile in man, early clinical results with MK0591 have been disappointing[3].

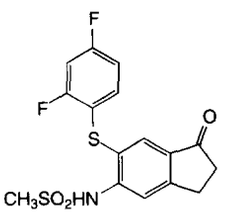
5-Lipoxygenase and FLAP inhibitors attenuate the formation of both peptidoleukotrienes and LTB_4, but have no effect on the formation of prostaglandins. Dual inhibitors have been identified, however, that affect both the cyclooxygenase and 5-lipoxygenase pathways, thus leading to a reduction in both prostaglandin and leukotriene products[12]. Typical among such compounds is WY47288 (Figure 2), which shows anti-inflammatory activity in animal models[13]. Unless such compounds show selectivity for COX-2 over COX-1 it seems likely that any additional benefit conferred will be moderated by gastric toxicity. Topical application should avoid such problems, but WY47288 was insufficiently potent by this route[12].

Selective leukotriene antagonists have also been developed by a number of groups. Many antagonists of the peptidoleukotrienes are now known, and a number have shown clinical benefit in asthmatics. Zafirlukast (Figure 2), which is effective and well tolerated in man[14,15], is the first compound of this type to have reached the market outside of Japan and is now available, or awaiting approval, in many countries[16]. In common with zileuton, zafirlukast is being positioned as a first-line prophylactic anti-asthmatic agent to compete with nedocromil and inhaled steroids[16]. Extended use of both agents will be needed to establish their relative effectiveness as anti-inflammatory anti-asthmatic agents, but the complex nature of asthma suggests that their greatest utility may well be in combination with other agents.

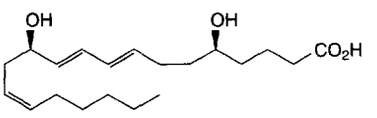
Various selective inhibitors of LTB_4, many designed on the basis of LTB_4 itself, have also been developed as potential anti-inflammatory agents for the treatment of psoriasis and asthma[3]. Most of these compounds suffered from poor pharmacokinetics or were poorly tolerated, but several compounds are still under clinical evaluation for inflammatory disorders[3,17]. LY293111 (Figure 2) is typical of those compounds undergoing further evaluation in psoriatic patients[18,19]. How important LTB_4 antagonists will be in inflammatory disorders is difficult to predict because many mediators other than LTB_4 are also involved in the



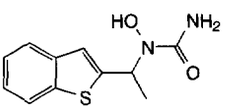
Celecoxib



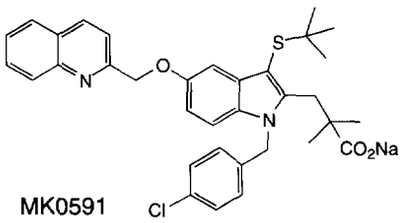
MK966



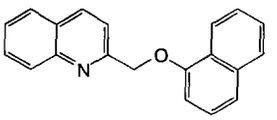
LTB_4



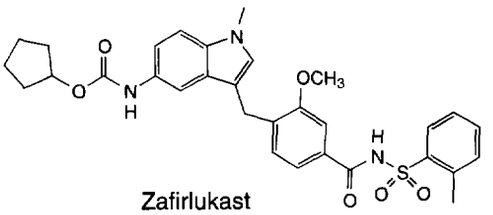
Zileuton



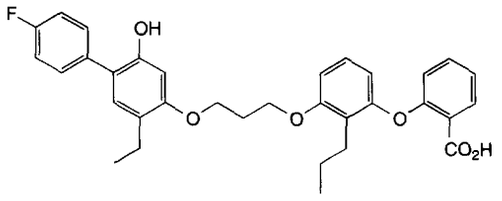
MK0591



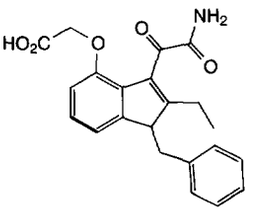
WY47288



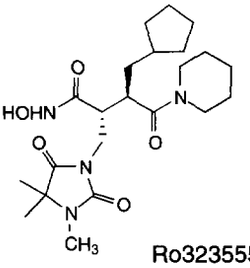
Zafirlukast



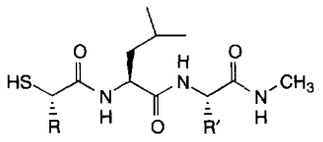
L293111



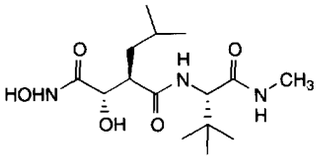
LY315920



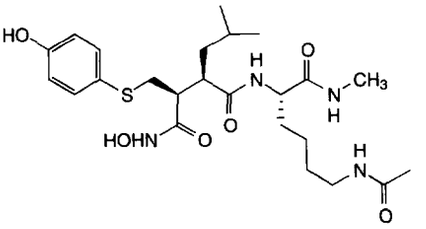
Ro323555



D5410 (R, R′ undefined)



Marimastat



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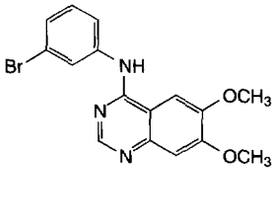

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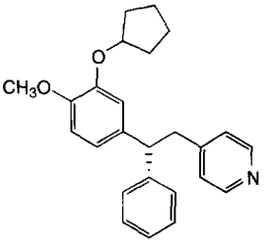
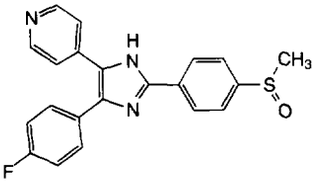


CDP840



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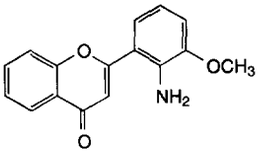

PD98059

Figure 2. *Structures of the compounds discussed in the text.*

chemotaxis of inflammatory cells (e.g. chemokines, see below).

Phospholipase A_2 inhibitors

In addition to inhibition of the enzymes responsible for the metabolism of released arachidonic acid, many workers have focused on inhibitors of phospholipase A_2 (PLA_2), the enzyme responsible for its release from phospholipid stores[12,20]. The inhibition of PLA_2 should theoretically attenuate all oxidative metabolites of arachidonic acid, including some potentially beneficial cyclo-oxygenase products. While such approaches early in the metabolic cascade address some of the issues levelled against single mediator targets, especially for complex diseases such as asthma, the full implications of such a wide-ranging inhibitory profile are far from understood. It is now clear, however, that several distinct PLA_2 isoenzymes exist[21–24], and it seems probable that selective inhibitors of specific isoenzymes may well confer benefits not inherent in broad-spectrum inhibitors. Potent inhibitors of the 14 kDa human non-pancreatic secretory PLA_2, which is elevated in the synovial fluid of arthritic joints and in the serum of patients suffering from septic shock or multiple injuries, have been identified through high-throughput screening[25,26]. From early leads the indole LY315920 (Figure 2) was identified for clinical evaluation[27]. The availability of the X-ray structure of this enzyme[28] should help in the identification of other potent inhibitors with which to establish the therapeutic potential of this compound class.

Over and above modulators of the arachidonic acid cascade, major advances in our understanding of the fundamental host response mechanisms to inflammation highlight many alternative drug targets through which greater efficacy and specificity of action may be achieved. Particularly significant is the detailed structural information available on many of these targets through the increased number and diversity of refined high-resolution X-ray crystallographic studies now reported. Because of the plethora of perceived opportunities this article focuses on selected targets, rather than attempting the impossible task of providing an exhaustive coverage.

Inhibitors of matrix metalloproteases

Remodelling of the extracellular matrix is an essential homeostatic process required for the maintenance of tissue integrity, but as is the case for many processes, an imbalance in the controlling mechanisms can have serious implications. In arthritic conditions, for example, chronic cartilage breakdown leads ultimately to joint destruction, severe pain and loss of joint function. Targeting collagenase 1 [also called matrix metalloprotease 1 (MMP-1)], the major zinc metalloenzyme responsible for cartilage breakdown, has led to the identification of many substrate-based inhibitors showing high potency and efficacy in animal models[29]. Most potent inhibitors of collagenase 1 and other matrix-degrading enzymes are derivatives of hydroxamic acid, a powerful bidentate zinc-complexing ligand[30,31]. Ro323555 (Figure 2) is representative of this compound class, differing from many earlier compounds by its oral activity and high selectivity for collagenase 1. Ro323555 is now in Phase II/III clinical trials for arthritis[32]. The thiolacetate D5410 (in Phase I trials) (Figure 2), one of a series of structurally related compounds[33], is also claimed to be orally effective[34]. The most advanced compound, marimastat (BB2516; Figure 2), another orally active agent having a broad inhibitory profile against matrix-degrading enzymes, has been extensively evaluated in man, and because it inhibits the enzymes responsible for tumour-cell migration holds promise as an inhibitor of metastasis[29,35–37]. The relative merits of inhibiting one or more matrix metalloproteases have been extensively debated[38], but until more prolonged clinical trials have been carried out this issue is likely to remain unresolved.

Inhibition of chemokines and cytokines

The most common response of cells to pro-inflammatory signals is via membrane-bound receptors, which may either propagate the signal externally through the formation of soluble mediators, or internally (via second messengers) through dimerization or conformational change. A complementary pathway utilizes cytosolic or nuclear receptors to effect a more direct response on the genetic machinery of the cell. While both processes effectively amplify the message received, the precise response is largely dependent on both the stimulus and the responding cell. Typically, transmission of pro-inflammatory signals between cells is mediated by cytokines and chemokines, which are, therefore, an important focus for drug intervention (Figure 3).

Chemokines

The primary role of chemokines is as chemoattractants, the concentration gradient of a chemokine released by one cell acting as a homing signal for another[39]. In inflammatory responses, for example, IL-8 released by monocytes at the site of inflammation leads to the attraction of neutrophils.

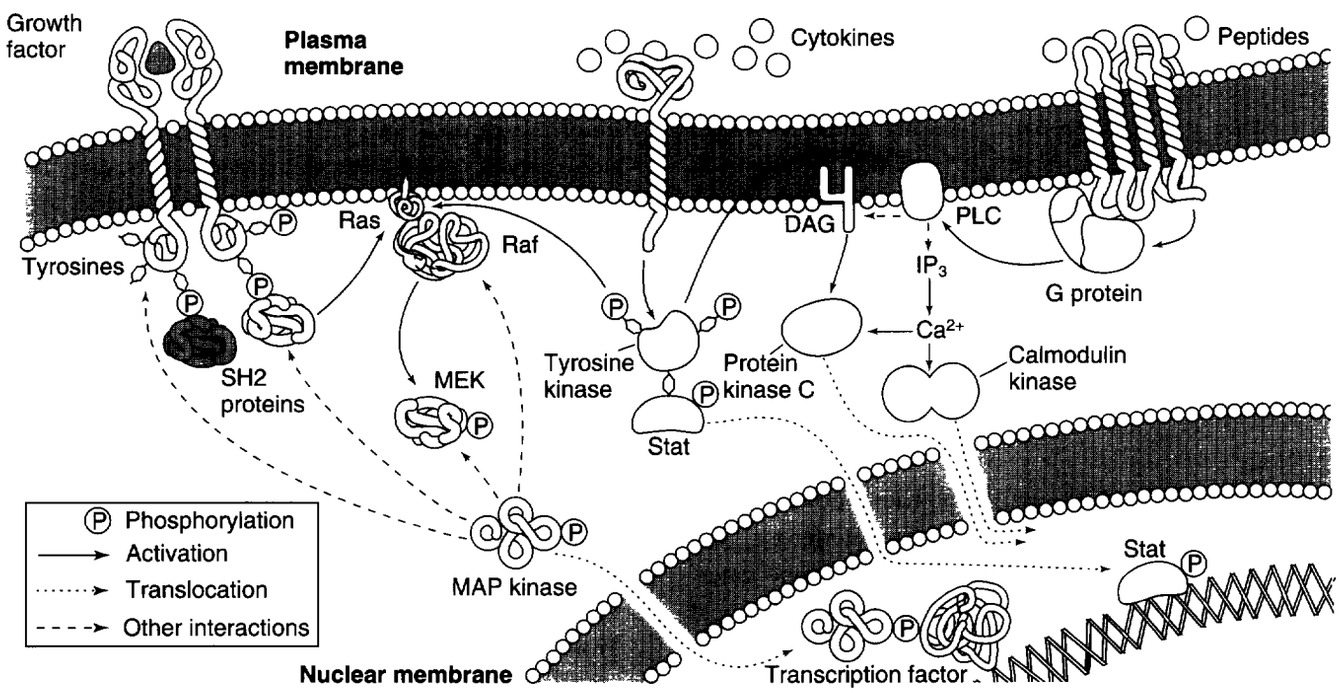

Figure 3. *Intracellular signalling mechanisms for inflammatory/immunomodulatory stimuli. Redrawn with permission from Ref. 84.*

Following the discovery of the first two members of the chemokine family, IL-8 and monocyte chemoattractant protein-1 (MCP-1), in the late eighties, it was hoped that selective immunoregulators would be found. With the discovery of many new members [the current total stands at around 30 chemokines and nine different seven-transmembrane G-protein-coupled receptors (GPCRs)] and the apparent redundancy in this signalling system, hope that this could be achieved waned[40]. However, recent animal studies indicate that a much greater cell-type selectivity exists in physiologically relevant systems[41]. Greater understanding of the interaction of chemokines with their receptors by comparison with other chemoattractants that interact with GPCRs has led to the recognition of two critical regions in the membrane receptor[42,43]. One is an area on the extracellular region that is involved with selective receptor recognition, and the second a region between the membrane and cytoplasm that triggers receptor activation. Some chemokine antagonists are known, but this new knowledge will extend the drug design opportunities open to medicinal chemists[44]. The recent recognition of a central role for chemokine receptors in facilitating cell infection by HIV (Ref. 45), together with the discovery of human eotaxin, an eosinophil-selective chemokine of importance in asthma[46], augur an exciting time for drug discovery in the chemokine field.

Cytokines

Targeting proteolytic events leading to the release of cytokines also provides a viable therapeutic approach to the modulation of inflammatory processes. Tumour necrosis factor α (TNF-α), for example, a potent pro-inflammatory and immunomodulatory cytokine implicated in such chronic diseases as rheumatoid arthritis and multiple sclerosis, is released from leukocytes by a specific enzyme, TNF convertase, for which small-molecule inhibitors are known[47–49]. While lacking selectivity for TNF convertase, several of these compounds [e.g. BB2116 (Figure 2)] are active in animal models, suggesting that therapeutic agents may soon be discovered[47,48]. The recent publication of the amino acid sequence for the human TNF convertase should facilitate the discovery of improved inhibitors[50,51]. Moreover, because many cell-surface proteins are known to be processed by similar proteolytic events[52,53], the possibility of extending this strategy to related inflammatory targets is becoming a real possibility.

A further cytokine, interleukin 1β (IL-1β), which plays an important role in joint destruction in rheumatoid arthritis and osteoarthritis, is released in a similar manner by a specific enzyme, interleukin 1β converting enzyme (ICE)[54]. This enzyme is a member of a growing family of novel cysteine proteases, other members of which are involved in programmed cell death (apoptosis)[54]. This family has a unique sequence preference for an aspartic acid residue on the N-terminal side of the cleavage site. The X-ray structure of human ICE has been published, and the first reports of peptidomimetics based on the natural substrate have appeared[55]. A number of companies are searching for nonpeptide leads using high-throughput screening technology.

Another group of cytokines from the haemopoietin superfamily, comprising IL-2, IL-4, IL-6 and the interferons, also plays an important role in inflammatory and immune states. All these cytokines have a basically similar mode of signal transduction. Released cytokines bind to extracellular receptors forming a complex consisting of several identical or different receptor subunits. Aggregation of these subunits brings together the intracellular chains leading to a new intracellular matrix, which can recruit further proteins, such as kinases, which propagate cellular activation[56,57]. In this way extracellular cytokines that are unable to penetrate cells transmit their stimulus to the nucleus of the activated cell. Attempts to find small molecules capable of inhibiting cytokine binding have met with little success, although one group has identified a 20-amino-acid inhibitor of cytokine binding using random phage display methodologies for lead generation[58]. More recently the discovery of specific kinases that can phosphorylate particular tyrosine residues on STAT (signal transducers and activators of transcription) proteins, thereby causing them to dimerize, translocate to the nucleus and initiate gene transcription, has focused attention on intracellular interactions as a source of novel inhibitors[59]. The race is on to identify selective cytokine inhibitors based on preventing this crucial dimerization step.

Inhibitors of protein tyrosine kinases

Dimerization of STATs is brought about by specific regions, termed SH2 domains, present on each STAT, which selectively identify phosphorylated tyrosines in the context of adjacent amino acids on a dimerization partner. Many different SH2 domains are known and they play important roles in various intracellular protein–protein interactions, such as kinase recruitment to complexed intracellular receptors. They are consequently a very active area for the discovery of therapeutic agents. The specific response of the immune system to foreign bodies (antigens) such as bacteria, viruses, animal dander or transplanted organs for example, is carried out by T cells, B cells and mast cells, which carry specific proteins to identify invading matter. Antigen-binding to T cells, for example, leads to the formation of a large complex consisting of a number of receptor subunits that become phosphorylated on a specific intracellular double tyrosine motif. This recruits a new tyrosine kinase, ZAP-70, possessing a tandem SH2 domain that specifically recognizes the double phosphotyrosine motif, and is essential for successful T-cell activation. The X-ray structure of this interaction has been published recently[60]. Inhibitors of this and similar interactions in B cells and mast cells are being investigated by a number of groups in the hope of discovering novel therapeutics for autoimmune disease, transplantation and asthma.

Protein tyrosine kinases (PTKs) play a critical role in a number of surface-receptor signal transduction pathways. Their overexpression, or constitutive activation by specific mutation, is commonly seen in cancer cells. Selective inhibition of the ATP cofactor binding cleft at the catalytic site of a number of these kinases is a very active area of anticancer research. Despite the conservation of specific residues at the ATP site, very selective and potent inhibitors have been identified. PD153035 (Figure 2), for example, is a picomolar inhibitor of the epidermal growth factor (EGF) receptor kinase and shows good selectivity against other kinases[61]. This EGF receptor is implicated in squamous cell, breast and ovarian cancer. Other PTK anticancer targets include platelet-derived growth factor and the intracellular PTK Abl (Refs 62,63). Because selective inhibition of specific PTKs has been demonstrated by a number of groups, present work is directed at improving *in vivo* activity of inhibitors[63]. Attempts to block the more diffuse substrate binding site have been less successful to date.

Phosphodiesterase inhibitors

Increased levels of the second messenger cAMP in inflammatory and immune cells oppose the activation induced by chemokines and cytokines, leading to an overall suppression of cell function. Because the cAMP activity in these cells is predominantly controlled by a specific hydrolytic enzyme (type 4 cyclic nucleotide phosphodiesterase, PDE 4)[64,65], inhibitors of this enzyme constitute an important anti-inflammatory and immunomodulatory opportunity[66,67].

While four PDE 4 isoforms are now known, in addition to a large number of spliced variants, the importance of these has yet to be established and there are no reports of subtype-selective inhibitors[68]. Several highly potent and selective inhibitors of PDE 4 are known, and some, such as CDP840 (Figure 2), reached Phase II clinical trials[69] before failing on efficacy grounds[70]. A particular problem with this approach has been the separation of beneficial activity from adverse central nervous system and gastrointestinal effects, but the identification of a specific isoform of PDE 4 postulated to be responsible for nausea and emesis suggests that better tolerated agents can be designed[71,72]. Compounds showing adequate selectivity for the inflammatory PDE 4 isoform compared with that responsible for emesis have been reported[73], but confirmation in man is still awaited.

Inhibition of other intracellular kinases

In addition to cAMP, complex serine and threonine kinase cascades are involved in intracellular signalling, which until recently have been difficult to unravel. Two inhibitors, SB203580 and PD98059 (Figure 2), which selectively block specific components of inflammatory signalling pathways, are important tools for dissecting these phosphorylation events, and have led to the identification of novel molecular targets. Thus, SB203580 and related compounds are unique in their ability to selectively suppress the secretion of pro-inflammatory cytokines such as IL-1 and TNF-α from inflammatory cells, doing so by potent and selective inhibition of a specific kinase[74]. More importantly, however, inhibitors of this type show activity in various models of acute and chronic inflammation[75], although no clinical data have yet been reported.

Nuclear receptor modulators

Corticosteroids are undisputedly the most effective anti-inflammatory agents, owing their activity to a regulatory effect on gene expression through interaction at specific nuclear receptors. Other agents affecting gene expression such as retinoids, vitamin D and thyroid hormone also exert their actions through nuclear hormone receptors[76]. Of recent interest are peroxisome proliferator-activated receptors (PPARs), for which natural ligands have only just been identified[77]. Implicated in lipid, cholesterol and lipoprotein metabolism, PPARs are potentially attractive drug targets, especially now that LTB_4 (see above) appears to be a natural ligand for PPARα (Ref. 77). Interestingly, both leukotrienes and prostaglandins interact at cell-surface receptors, so the further identification of intracellular receptors opens up a host of new drug discovery opportunities. Moreover, X-ray crystallographic studies of the ligand binding domains of several nuclear hormone receptors offer a complementary approach to inhibitor design and a rational means to ligand optimization. Such studies provide a much broader opportunity for the regulation of nuclear receptors for which small molecules are the natural ligands, and to the specific treatment of diverse inflammatory disorders. Interaction at PPARα receptors raises a number of complex yet important questions that still have to be answered[78], and detailed evaluation of selective agents in animals and man is necessary to provide a better understanding of their physiological role.

The Rel/nuclear factor-κB (NFκB) family of transcription factors has also proved an attractive target for the design of inhibitors. These agents play a primary role in the regulation of immune and inflammatory responses provoked by IL-1, TNF-α, lipopolysaccharide, UV light and antigen responses, and are also subverted by a number of viruses during infection[79,80]. Increased understanding of the activation of this family of transcription factors suggests a number of new approaches to the design of inhibitors for this signalling pathway. NFκB is found in the cytoplasm as a complex with an inhibitory protein I-κB. Activation of NFκB and translocation to the nucleus requires phosphorylation of I-κB followed by site-specific ubiquitinylation and consequent degradation by proteasomes[81]. NFκB, which exists as a hetero- or homodimer, then translocates to the nucleus, where it binds to DNA. X-ray crystallography[82] and photocrosslinking studies[83] have identified amino acids in NFκB that are involved in DNA binding, and give increased understanding of the various recognition sites for different homo- and heterodimer transcription factors. Each of these steps in the activation of NFκB and subsequent gene transcription is under investigation by a number of companies as novel targets for the discovery of new anti-inflammatory and anti-cancer leads, despite offering a significant challenge to the medicinal chemist.

Conclusion

With such a plethora of new, well-defined molecular targets it is reasonable to expect the discovery of new anti-inflammatory drugs differing in their mechanism of action from existing therapies. Only time will tell whether significant advantages are offered by these alternative approaches, but early clinical data in some instances clearly suggest an

optimistic forecast. It is also likely that rapid advances in genomics will lead to an increasing array of novel targets, ultimately providing hope for more effective anti-inflammatory and immunomodulatory treatments.

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