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Respiratory diseases

New aspects of p38 mitogen activated protein kinase (MAPK) biology in lung inflammation

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Lung inflammation features in asthma, chronic obstructive airways disease (COPD), acute respiratory distress syndrome (ARDS), cystic fibrosis (CF) and others. Whilst in asthma anti-inflammatory glucocorticosteroids are generally effective, certain individuals are steroid resistant and in COPD, ARDS and CF, as well as disease exacerbations caused by infection, there seems little benefit. We summarise recent advances in p38 mitogen activated protein kinase (MAPK) biology and document beneficial and possibly detrimental effects in respect of lung inflammation.

Introduction

In asthma, COPD, ARDS and other inflammatory diseases, the upregulation of cytokines, chemokines and other proteins leads to the recruitment and influx of inflammatory cells. The p38 mitogen activated protein kinase (MAPK) plays a key role in these processes via ligands binding to receptors or cellular stresses (Box 1). Small G-proteins then activate MAPK kinase kinases (MAP3K), which phosphorylate, and activate, the MAPK kinases (MKKs) (Fig. 1). MKK6 activates all four (α , β , γ , δ), whereas MKK3 activates the α and β p38 MAPK isoforms. Downstream targets are numerous and play roles in the regulation of inflammation via transcriptional, post-transcriptional, translational and other targets (Fig. 1).

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Finally, in understanding p38 MAPK biology, it is important to appreciate that the commonly available inhibitors are selective for p38 α and p38 β , not p38 γ and p38 δ , and our current knowledge of targets (Fig. 1) and responses (Tables 1 and 2) reflects this.

Activators of the p38 MAPK

Activation of p38 MAP kinases by cellular stresses and inflammatory cytokines, such as TNF α and IL-1 β , is well established (Fig. 1) [1,2]. In addition, newer stimuli, for example, the pro-inflammatory cytokine, IL-17, which induces IL-8 synthesis (see [2]), IL-18, which primes neutrophil functions [3], or IL-25, a novel Th2 cytokine that upregulates cytokine and chemokine expression from eosinophils [4], are continually being described (Box 1). However viruses and bacteria, as the causative agents of pulmonary diseases, along with their products, also activate the p38 MAPK and are increasingly coming to light as principal causes of exacerbations in asthma and COPD [2,5].

Thus respiratory viral infections, for example, human rhinovirus (HRV) and respiratory syncytial virus (RSV), upregulate the expression of multiple cytokines (IL-1, TNF α , IL-6, G-CSF, GM-CSF) and chemokines (IL-8, ENA-78, GRO α , RANTES) in epithelial cells, as well as ICAM-1 in endothelial cells, via the

Box 1. Stimuli and signals relevant to lung inflammation that activate the p38 MAPK

Cytokines & chemokines

IL-1 (IL-1 α , IL-1 β), TNF α , IL-8, IL-17, IL-18, IL-25, eotaxin, MIP1 α

Growth factors

EGF, PGDF, TGF β

Neurotransmitters, kinins and others

Substance P, bradykinin, acetylcholine (methacholine) adenosine, fMLP

Lipids and related products

Platelet activating factor (PAF), leukotrienes (LTC₄, LTD₄)

Stresses

Oxidative stress (ROS), stretch, hyperosmolarity, hypoxia

Infection

Bacterial products (LPS, peptidoglycans), *Burkholderia pseudomallei*, *Burkholderia cenocepacia*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, Influenza virus (IV), respiratory syncytial virus (RSV), adenovirus, human rhinovirus (HRV), SARS virus.

Other

Diesel exhaust/carbon particulates, cigarette smoke, CO (carbon-monoxide), thrombin, ECM components, ICAM-1 ligation, IgE/Fc ϵ RI ligation, CD40 ligation, serum, phorbol esters, dsRNA

p38 MAPK activating stimuli listed are taken from the current review and references [1,2]. The present list is therefore not exhaustive.

p38 MAPK pathway [5–8]. Activation of p38 MAPK by influenza virus (IV), including the H5N1 ‘bird flu’ strain, suggests a similar induction of cytokines and chemokines [9]. These effects are likely to be mediated via the MAP3K, apoptosis signal-regulating kinase (ASK) 1, which is also responsible for IV-induced apoptotic cell death [10]. Furthermore, the finding that double stranded RNA (dsRNA), which acts via the dsRNA-dependent protein kinase (PKR) and mimics responses to many RNA viruses including RSV, HRV and IV, suggests that these are general responses to viral infection [5]. In addition, HRV infection of alveolar macrophage and monocytic cells leads to p38 and activating transcription factor (ATF)-2 phosphorylation as well as the induction of MCP-1 expression [11]. Therefore, despite not affecting HRV replication, IV infection, or IV and severe acute respiratory syndrome (SARS) viral protein synthesis, these data indicate the therapeutic potential of targeting p38 MAPK in epithelial and macrophage cells in the context of virus-induced inflammation and apoptosis [6,9,10,12].

Similarly, the bacterial product, lipopolysaccharide (LPS), is a potent inducer of p38 MAPK and in LPS-induced ARDS, this pathway plays a role in both bronchoconstriction and neutrophil recruitment [2,13]. Numerous other bacterial pathogens also induce pulmonary inflammatory responses via p38 MAPK. For example, *Burkholderia pseudomallei*, the causative agent of melioidosis, activates p38 MAPK and this appears necessary for epithelial cell invasion [14]. *Burkholderia*

cenocepacia, a pathogen that causes fatal pulmonary disease in the immunocompromised and CF sufferers, induces expression of the bradykinin B1 kinnin receptor, and presumably other inflammatory mediators, via a p38-dependent mechanism in fibroblasts [15]. Likewise, *Streptococcus pneumoniae*, the predominant cause of community-acquired pneumonia, and a major cause of death by infectious disease in industrialised countries, induces IL-8 expression via the p38 pathway [16]. This is also true for cell fractions and lipopeptide, from non-typeable *Haemophilus influenzae*, which is a major cause of COPD exacerbation [17].

In addition, several novel inflammatory compounds, including pollutants, such as ultrafine carbon particulates and cigarette smoke [18,19], lipid mediators, such as leukotriene C₄ (LTC₄) and LTD₄ [20,21], as well as mechanical stretch or cholinergic stimulation [22], all activate the p38 MAPK and might therefore contribute to inflammatory processes. Interestingly, the response to cigarette smoke was synergistically increased by heat-inactivated bacteria suggesting the possibility of combinatorial effects in diseases such as COPD [19].

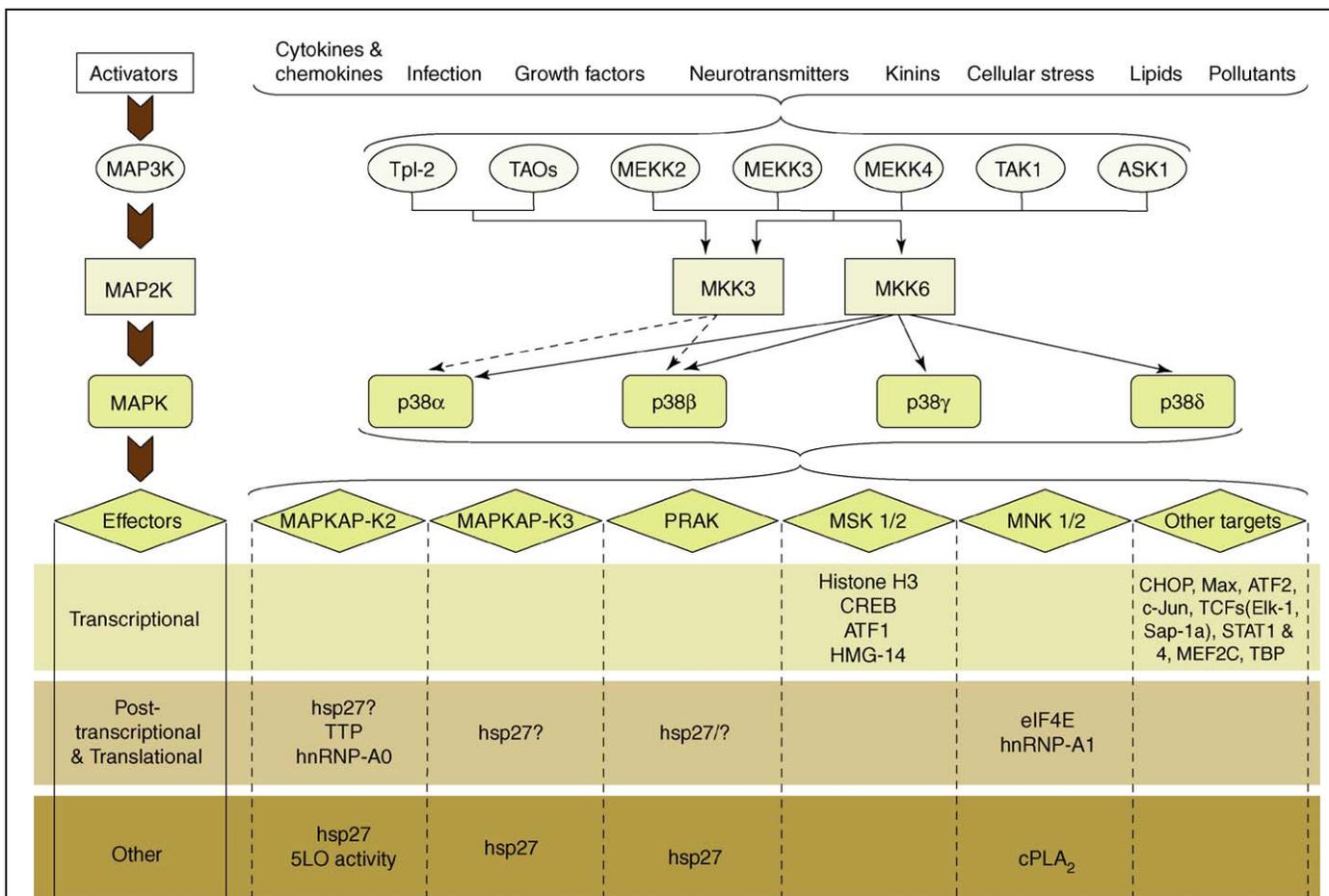
p38 MAPK in inflammatory gene expression

Transcriptional responses

The p38 MAPK regulates gene transcription via phosphorylation of numerous transcription factors (Fig. 1). In addition, the activity of AP-1, a major positive regulator of inflammatory genes, is also enhanced by increasing the expression of the constituent proteins, c-Jun and c-Fos. In this case, p38-dependent phosphorylation of ternary complex factors (TCFs) promotes interaction with serum response factor (SRF) to drive transcription from serum response elements (SREs), such as are found in the *c-fos* promoter [1,2]. Alternatively, effects mediated via adenosine- and uridine- (AU)-rich elements (ARE) in the 3' untranslated region (UTR) of *c-fos* and *c-jun* can also enhance expression (see below).

p38 MAPK can also act downstream of transcription factor DNA binding [2]. Thus, p38 MAPK potentiates the transcriptional competency, not DNA binding, of the inflammatory transcription factor, NF- κ B, via processes that may also determine differential responsiveness of NF- κ B-dependent genes [2]. Similarly, p38 inhibitors prevented the *S. pneumoniae*-dependent induction of IL-8 and GM-CSF from bronchial epithelial cells by blocking NF- κ B-dependent transcription and phosphorylation, but not nuclear translocation or recruitment to the promoter [16].

Glucocorticosteroid control of asthma occurs via the glucocorticoid receptor (GR). However, in some patients clinical utility is limited by steroid-insensitivity, a phenomenon that may involve phosphorylation of GR by MAPKs and reduced anti-inflammatory ability (see [2]). Certainly, p38-dependent phosphorylation of GR diminishes GR-dependent transcriptional responses [23], which given a role for steroid-inducible



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Figure 1. The p38 mitogen activated protein (MAP) kinase (MAPK) activation cascade. A Schematic diagram showing activation pathways leading to the p38 MAPK isoforms. Various stimuli, stresses or other insults acting on the cell may cause activation of p38 MAPK isoforms through the sequential activation of the MAP kinase kinases (MAP3K), which phosphorylate and activate the MAP kinase kinases (MAP2K). These in turn phosphorylate the p38 MAPKs, which on activation, are able to phosphorylate several targets including downstream kinases as well as other effectors. ARE, AU response element; ASK1, apoptosis signal-regulating kinase 1; ATF, activating transcription factor; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; cPLA₂, cytosolic phospholipase A₂; CREB, cyclic AMP response element binding protein; eIF4E, eukaryotic initiation factor 4E; HMG-14, high mobility group 14; hsp27, heat shock protein 27; MAPK, mitogen-activated protein kinase; MAP2K, mitogen-activated protein kinase kinase; MAP3K, mitogen-activated protein kinase kinase kinase; MAPKAP-K (or MK), MAPK-activated protein kinase; MEK2C, myocyte-specific enhancer binding factor 2C; MEKK, mitogen-activated protein kinase/extracellular protein kinase kinase kinase; MKK, MAP kinase kinase; MNK, MAPK-interacting kinase; MSK, mitogen- and stress-activated protein kinase; PRAK, p38-related/activated protein kinase; STAT, signal transducer and activator of transcription; TAK, TGFβ activated kinase; TAOs, thousand and one kinases; TBP, TATA-binding protein; TTP, Tristetraprolin, TCF, ternary complex factor; SLO, 5-lipoxygenase; hnRNP, heterogenous nuclear ribonuclear protein.

Table I. Consequences of p38 inhibition that may be beneficial in the context of lung inflammation

Cell type	Response targeted	Expected outcome of targeted inhibition of p38 MAPK ^a	Who is working on this response and when? ^b	Refs
Eosinophil	Chemotaxis and degranulation following OVA ^c and leukotriene challenge	Reduction of eosinophilia, the respiratory burst and release of ECP ^d , EDN ^e .	Kampen GT, Alam R. (2000) Lynch OT, Lindsay MA. (2001) Adachi T, Alam R. (2000)	[2]
Neutrophils	Degranulation	Reduced release of neutrophil derived inflammatory proteins	Smolen JE, Simon SI. (2000)	[2]
Neutrophils	Granule production	Reduced release of neutrophil derived inflammatory proteins	Underwood DC, Griswold DE. (2000)	[2]
Neutrophils	Acute neutrophil influx to the lung following OVA sensitisation	Reduced neutrophil influx to the lung	Taube C, Gelfand EW. (2004)	[34]

Table 1 (Continued)

Cell type	Response targeted	Expected outcome of targeted inhibition of p38 MAPK ^a	Who is working on this response and when? ^b	Refs
Epithelial cells	Cytokine, chemokine, prostaglandin, and receptor expression	Reduced expression of IL-1 ^l , TNF α ^g , IL-6, G-CSF ^h , GM-CSF ⁱ , IL-8, ENA-78 ^j , GRO α ^k , RANTES ^l , neutrophil mobilising cytokines, PGE ₂ ^m , and the bradykinin B1 kinnin receptor	Matsumoto K, Horie T. (1998) Hashimoto S, Horie T. (2000) Laan M, Linden A. (2001) Newton R, Barnes PJ. (2000) Phagoo SB, Warburton D. (2005)	[2] [2] [2] [2] [15]
Epithelial cells	Invasion of <i>Burkholderia pseudomallei</i>	Reduced invasion into epithelial cells	Utainsincharoen P, Sirisinha, S. (2005)	[14]
Epithelial cells	LTC ₄ ⁿ induced TGF β ^o and airway remodeling	Reduced expression of TGF β and therefore lower fibroblast proliferation	Perng DW, Lee YC. (2005)	[20]
Epithelial cells	Downregulation of ENaC ^p by IL-1 β	Reduced downregulation of ENaC may maintain salt and water flow into the epithelium and prevent lung oedema	Roux J, Pittet JF. (2005)	[36]
Goblet cells	Mucous production	Reduced MUC5A ^q production	Yoon JH, Song KS (2002)	[2]
Airway smooth muscle cells	Cytokine production	Reduced expression of eotaxin	Hirst SJ, Lee TH (2002)	[2]
Airways smooth muscle	Cell migration in response to PDGF ^r , IL-1 β , TGF β via hsp27	Reduced migration	Hedges JC, Gerthoffer WT. (1999)	[2]
Endothelial cells	Cytokine, chemokine, adhesion molecule, protease and prostaglandin expression	Reduced expression of IL-8, MCP-1 ^s , IL-6, ICAM ^t -1, prostaglandin, MMP ^u expression, cell efflux and oedema formation	Hashimoto S, Horie T. (2000) Goebeler M, Ludwig S. (1999) Ridley SH, Saklatvala J. (1997)	[2]
Mast cells	Cellular migration and chemokine expression	Inhibition of antigen/FC ϵ R1 induced chemotaxis and IL-8 release	Ishizuka T, Mori M. (2001) Feoktistov I, Biaggioni I. (1999)	[2]
Lymphocytes	Cytokine expression	Inhibition of IL-5 and IL-13	Mori A, Akiyama K. (1999) Chen CH, Ray A. (2000)	[2]
Monocytes	Monocyte differentiation and chemotaxis	Inhibition of macrophage maturation and influx of inflammatory cells	Ayala JM, Hanlon WA. (2000)	[2]
Monocyte/macrophage	Production of inflammatory proteins	Inhibition of MIP-2 ^v , TNF α , COX-2 ^w , and GM-CSF ⁱ expression	Nick JA, Worthen GS. (2000) Meja KK, Giembycz MA. (2000) Niino H, Niho Y. (1998)	[2]
Multiple cells	ARE ^x -dependent mRNA stabilisation	Reduced stability of ARE containing mRNA	Clark AR, Saklatvala, J (2003)	[24]
Multiple cells	Glucocorticoid resistance	Increased Glucocorticoid-dependent transcription	Szatmary Z, Vilcek J. (2004) Irusen, E, Adcock IM. (2002)	[23] [2]

^a MAPK: mitogen activated protein kinase.

^b All authors are listed as first and last authors followed by year of publication.

^c OVA: ovalbumin.

^d ECP: eosinophil cationic protein.

^e EDN: eosinophil-derived neurotoxin.

^f IL: interleukin.

^g TNF: tumour necrosis factor.

^h G-CSF: granulocyte colony-stimulating factor.

ⁱ GM-CSF: granulocyte macrophage colony-stimulating factor.

^j ENA-78: epithelial neutrophil-activating peptide-78.

^k GRO: growth related oncogene; hsp, heat shock protein.

^l RANTES: regulated on activation normal T cell expressed and secreted.

^m PG: prostaglandin.

ⁿ LT: leukotriene.

^o TGF: transforming growth factor.

^p ENaC: epithelial sodium channel.

^q MUC: mucine.

^r PDGF: platelet-derived growth factor.

^s MCP: monocyte chemoattractant protein.

^t ICAM: intercellular adhesion molecule.

^u MMP: matrix metalloproteinase.

^v MIP: macrophage inflammatory protein.

^w COX: cyclooxygenase.

^x ARE: AU rich element.

Table 2. Consequences of p38 inhibition that may not be beneficial to the resolution of lung inflammation

Cell type	Response targeted	Expected outcome of targeted inhibition of p38 MAPK ^a	Who is working on this response and when? ^b	Refs
Multiple cell types	Decrease in ARE ^c containing mRNA stability via production and activation of TTP	Increased expression of pro-inflammatory proteins.	Tchen CR, Clark, AR. (2004)	[31]
Multiple cell types	Induction of SOCS3 ^d	Increased expression of Th ₂ cytokines	Canfield S, Rothman P. (2005)	[32]
Multiple cell types	Inhibition of TLR2 ^e expression	Increased activation of the TLR2 pathway	Imasato A, Li JD. (2002)	[30]
Multiple cell types	Repression of the Ras, MEK1 ^f and ERK ^g 1/2 pathways by activation of the phosphatases PPI ^h and PP2A	Increased activation of pro-inflammatory pathways	Westermarck J, Kahari VM. (2001)	[2]
Smooth muscle cells	Inhibition of cyclin D1	Increased cellular proliferation	Page K, Hershenson MB. (2001)	[2]
Monocytes/macrophages	Cytokine Expression	Decreased expression of IL-10 ⁱ and IL-12	Niino H, Niho Y. (1998) Choudhury BK, Sur S. (2002)	[2] [39]

^a MAPK: mitogen activated protein kinase.

^b All authors are listed as first and last authors followed by year of publication.

^c ARE: AU rich element.

^d SOCS: suppressor of cytokine signalling.

^e TLR: toll-like receptor.

^f MEK: MAPK/ERK kinase.

^g ERK: extracellular kinase.

^h PP: protein phosphatase.

ⁱ IL: interleukin.

genes in the anti-inflammatory actions of glucocorticosteroids [24], points to an involvement in steroid resistance.

Post-transcriptional roles

The characterisation of cytokine-suppressive anti-inflammatory drugs (CSAIDs) (p38 inhibitors) revealed inhibition of cytokine biosynthesis via post-transcriptional and translational mechanisms [1,2]. This involved the downstream kinase, MAPK activated protein kinase 2 (MAPKAP-K2) and is particularly relevant for genes, such as TNF α , that contain AREs in their 3'UTRs (Table 3) [24]. Indeed, many inflammatory genes contain one or more ARE (Table 3), and p38-dependent mRNA stabilisation therefore regulates inflammatory and virally induced gene expression [7,24]. Despite the identification of numerous ARE-binding proteins (ARE-BPs) [25] (Fig. 2), there is considerable uncertainty as to targets of the p38 pathway [26]. This is being said, that binding of heterogenous nuclear ribonuclear protein (hnRNP) A0 to the TNF α , COX-2 and MIP-2 3'UTRs is MAPKAP-K2-dependent,

blocked by the p38 inhibitor, SB203580, and correlates with mRNA stability [25]. Similarly, binding of hnRNP A1 to the TNF α ARE increases following phosphorylation by MNK [27], kinases which are also implicated in translation via phosphorylation of the eukaryotic initiation factor 4E (eIF4E) (see[2,24]) (Fig. 2). Furthermore, Mnk knock-down, or pharmacological inhibition, reduces TNF α expression and supports a role for this pathway [27]. Further complexity is introduced as p38-dependent stabilisation of certain ARE-containing mRNAs (e.g. COX-2, TNF α) occurs via blocking deadenylation [28] to promote both mRNA stability and translation which are themselves coupled to poly-A tail length (Fig. 2). Thus poly-A tail shortening reduces translation efficiency, precedes mRNA degradation and can be targeted by the p38 MAPK to regulate inflammatory gene expression [24,28] (Fig. 2).

Modulation of, and by, p38 MAPK

The p38 MAPK is subject to inhibition by three main classes of phosphatase [24]. In this context, the dual-specificity phosphatase, MAPK phosphatase 1 (MKP-1), is rapidly induced by pro-inflammatory stimuli, for example LPS on alveolar macrophage [29]; to terminate p38 signalling [24]. MKP-1 is also induced by glucocorticosteroids and this illustrates an anti-inflammatory mechanism by which steroid-dependent transcription prevents biosynthesis (transcriptional, post-transcriptional and translational in Fig. 1) of inflammatory mediators [24,29]. Furthermore, cAMP-elevating agents also induce MKP-1 raising the possibility of a role in the anti-inflammatory effects of long acting β_2 -agonists and other cAMP-elevating drugs [24].

Table 3. Classes of AU-rich elements (ARE)

Class	Structure of element	Example genes
I	Scattered AUUUA plus U-rich context	c-fos, c-myc
II	Multiple clustered/overlapping AUUUA	GM-CSF, COX-2, TNF α , IFN α , IL-2
III	U-rich AREs that lack AUUUA motifs	c-jun

Note: Class II AREs can be further divided according to the number (1–5) of overlapping AUUUA motifs.



Figure 2. Post-transcriptional and translational control by adenosine-, uridine-, (AU) rich elements (AREs) in the 3'UTR. A schematic showing an ARE-containing mRNA that has bound eukaryotic initiation factor (eIF) 4E, which specifically recognises the cap structure (m^7Gppp), eIF4A, a single stranded RNA binding protein with helicase activity, and eIF4G, a protein that links the cap region to the 40S ribosome. The translation start and stop sites are shown, as is an ARE and the poly-A tail, which bind multiply copies of the poly-A binding protein 1c (PABP). A series of ARE-binding proteins (ARE-BP) are depicted and include the destabilisation protein TTP (tristetraprolin). Known ARE-BPs, along with their functions are listed below the ARE. Terminal kinases of the p38 mitogen activated protein kinase cascade (MAPK) are depicted with p38 MAPK phosphorylating and activating the downstream kinases MAPK activated protein kinase-2 (MAPKAP-K2 or MK2) and MAPK interacting kinase (MNK). p38 MAPK targets the ARE-BPs, hnRNP A0 and A1, via MK2 and MNK, respectively, and this may play a role in mRNA stabilisation. Other proteins that may be targeted by the p38 MAPK include PABP and other ARE-BPs. In addition, p38 MAPK may also target a deadenylase to prevent loss of the poly-A tail and promote mRNA stability and translation. This along with phosphorylation of eIF4E may facilitate association between the poly-A and the cap structure, an event that could be promoted by ARE-BPs, and lead to enhanced efficiency of translation. Finally, the p38 MAPK, acting via MK2, promotes TTP expression and also activates TTP by phosphorylation to exert negative feedback control and destabilisation of ARE containing mRNAs. Other abbreviations: AUF, AU-binding factor; BRF-1, butyrate-response factor 1; hn RNP, heterogeneous nuclear ribonuclear protein; TIA-1, T cell-restricted intracellular antigen-1 (TIA1-); TIAR, TIA-related protein.

One surprising consequence of reduced p38 activity, is that the induction of Toll-like receptor-2 (TLR2) by nontypeably *H. influenzae* (NTHi) is subject to p38-dependent feedback inhibition such that NTHi infection in the presence of glucocorticosteroids enhances TLR2 expression and signalling to increase the release of cytokines and chemokines [30]. Thus by inducing MKP-1 and inhibiting p38, glucocorticosteroids can enhance inflammatory responses to certain infections and this could also occur in the context of p38 inhibitors.

As noted above, binding of ARE-BPs to 3'UTRs imparts considerable regulatory control. Tristetraprolin (TTP) is one such protein, which is responsible for mRNA destabilisation and whose deletion elevates $TNF\alpha$ expression and leads to various inflammatory disorders [24]. This acute phase gene is

induced by pro-inflammatory stimuli and provides negative-feedback control [24]. Therefore, the inhibition of pro-inflammatory pathways could, by preventing TTP expression, stabilise and enhance the expression of ARE containing genes. In this context, the p38 pathway, acting via MAPKAP-K2 and the TTP ARE, stabilises TTP mRNA and p38 inhibition profoundly reduces TTP expression [31]. Furthermore, TTP destabilising activity might require p38-dependent phosphorylation and provides additional evidence that effects of the p38 MAPK on TTP might be desirable in inflammation [24].

Further key modulatory roles include the p38-dependent expression of the anti-inflammatory cytokine, IL-10, and the p38-dependent induction of suppressor of cytokine signal-

ling 3 (SOCS3) by IL-4, which might be important in Th2-dependent diseases such as asthma [2–32]. As SOCS3 limits signalling via gp130 cytokine receptors, the inhibition of p38 MAPK could lead to exaggerated responses and amplification of Th2-dependent disease.

Animal models and clinical aspects

Many of the responses above, for example, release of and responses to IL-8 [2,3,16,17], suggest a major effect on neutrophilic disease. Neutrophil influx is usually a hallmark of obliterative bronchiolitis following lung transplantation and using an *in vivo* rat model, a p38 inhibitor was shown to dramatically reduce inflammatory cytokines, tracheal occlusion and organ rejection [33]. This is consistent with data from LPS-challenged mice in which TNF α production was reduced by SB239063, a potent second-generation p38 inhibitor ([2] and see refs therein). Likewise in an ovalbumin (OVA) sensitisation and challenge mouse model, a p38 inhibitor again prevented neutrophil increases in the bronchoalveolar lavage (BAL) fluid [34]. Interestingly, whilst increased cytokine levels (for IL-4, -5, -12, -13 or interferon- γ) and goblet cell hyperplasia were unaffected, mice treated with p38 inhibitor revealed significantly decreased airways hyper-reactivity (AHR) [34]. Similarly, OVA-induced eosinophilic inflammation in both mice and guinea pigs was also prevented by SB239063 (see [2]). This compound reduced LTD₄-induced eosinophilia and promoted eosinophil apoptosis suggesting a beneficial effect in eosinophilic diseases, such as asthma. This conclusion also receives support from an OVA-induced mouse asthma model in which antisense oligonucleotides to p38 MAPK prevented pulmonary eosinophilia, AHR and mucus hypersecretion [35]. In addition, lung oedema is frequently associated with disease. This is promoted by the down-regulation of the epithelial sodium channel, ENaC α , to reduce water and ion transport into the tissues. As this process requires p38 MAPK, p38 inhibitors could aid the control of lung oedema [36].

In terms of infection and disease exacerbation, epithelial cells from COPD patients show enhanced, as well as additional (versus non-COPD patient), p38 MAPK-dependent inflammatory responses to *H. influenzae* [37]. Likewise CF tissues showed enhanced responses to *Pseudomonas aeruginosa* LPS via increased IL-8 release and neutrophil migration and therefore suggest a benefit from p38 inhibition in CF inflammation [38]. One possible worry in respect of p38 inhibition in infection relates to the ability of DNA containing unmethylated CpG, as occurs in the context of bacterial infection, to induce strong Th1-type immune responses and reduce the development of Th2 allergic asthma in a mouse model [39]. This effect, which might be a part of immunological education, requires the p38-dependent release of the pro-Th1 cytokine, IL-12, from alveolar macrophage. Thus the use of p38 inhibitors to combat inflammation in infection

could subsequently enhance the development of allergic disease. Another, frequently fatal disease with relatively rapid onset is idiopathic pulmonary fibrosis (IPF). This is poorly responsive to current treatments and is characterised by irreversible lung fibrosis, which is now reported to involve the p38 MAPK [40]. Given a role of p38 MAPK in the expression of growth factors and fibroblast functions [2], it is possible that p38 inhibition may prove to be of therapeutic benefit.

Summary and conclusions

The above examples reveal a critical role of the p38 MAPK in regulating inflammatory gene expression and suggest a key role in pulmonary disease. These effects occur by various transcriptional, post-transcriptional and translational mechanisms and the data presented supports the potential use of p38 inhibitors in controlling inflammatory responses. The fact that anti-inflammatory glucocorticoids target the p38 pathway, via the induction of MKP-1, supports the pharmacological rationale for targeting p38 MAPK in inflammation. Furthermore, the finding that the p38 MAPK targets GR to reduce responsiveness raises the possibility that p38 inhibitors could be used, not only in their own right as anti-inflammatory agents, but also in conjunction with glucocorticosteroids to improve patient sensitivity to these compounds. This effect could be particularly valuable in the context of steroid resistant or insensitive patients who often remain poorly controlled, if at all, and require high dose oral corticosteroids. In this context, disease progression in COPD is poorly responsive to glucocorticosteroids and pathogenesis involves the neutrophil. As p38 inhibitors appear effective at targeting neutrophil functions and many COPD triggers, for example cigarette smoke or particulates, promote neutrophil recruitment via the p38 pathway, inhibitors of the p38 pathway may show benefit in this disease. In addition, both viral and bacterial infections are potent inducers of the p38 MAPK and lead to significant health issues both arising directly from infectious disease as well as from the exacerbation of conditions such as asthma or COPD. Thus in situations where the underlying infection is controllable by other means, such as antibiotics or anti-virals, it is possible that p38 inhibition may be appropriate to deal solely with the resultant inflammatory response. Notwithstanding this positive outlook, it is to be noted that p38-dependent processes also take a significant role in feedback inhibition of inflammatory genes via the expression and activation of TTP. Removal of these control processes could tend to increase inflammatory gene expression. Thus the balance between these positive and negative effects can be stimulus- and gene-specific and will only become apparent following further studies. In addition, whilst there is some evidence that infection, for example of *B. pseudomallei*, might require p38 MAPK, this is less certain in respect of other infectious agents and this issue still requires

specific testing. Furthermore, the finding that TLR2 expression and signalling is enhanced by p38 inhibition, as a result of loss of feedback control, raises the possibility that p38 inhibitors can only be effective in the context of certain (non-TLR2-dependent) infections. Finally, loss of SOCS3 and IL-12 expression could both lead to enhanced Th2-dependent inflammatory responses and can, in time, lead to the development of elevated allergic reactions. In conclusion, these data, indicate a clear ability of p38 inhibitors to target inflammation, but certain questions remain as to potentially undesirable events that could limit the clinical utility of such compounds.

Acknowledgements

R.N. is a Canadian Institutes of Health Research (CIHR) New Investigator and Alberta Heritage Foundation for Medical Research (AHRMR) Scholar and holds start up and operating grants AHRMR and CIHR respectively.

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