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Pharmacology & Therapeutics 121 (2009) 1–13

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Targeting the NF-κB pathway in asthma and chronic obstructive pulmonary disease

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ARTICLE INFO

Keywords:
NF-κB
IKK-β
Asthma
COPD
Inflammation
Lung

Abbreviations:
COPD, chronic obstructive pulmonary disease
CS, cigarette smoke
GC, corticosteroid
NF-κB, nuclear factor-κB
AHR, airway hyperreactivity
ASM, airway smooth muscle
PEF, peak expiratory flow
FEV, forced expiratory volume
LAR, late asthmatic response
RV, rhinovirus
RSV, respiratory syncytial virus
FGF, fibroblast growth factor
VEGF, vascular endothelial growth factor
dsRNA, double stranded RNA
ssRNA, single stranded RNA
LPS, lipopolysaccaride
RHD, Rel homology domain
NLS, nuclear localisation sequence
IKK, I-κB kinase
NEMO, NF-κB essential modulator
RIG-I, retinoic acid inducible gene
MDA-5, melanoma differentiation associated gene-5
PKR, protein kinase R
TCR, T-cell receptor
RANKL, receptor activator of NF-κB ligand
HAT, histone acetyl transferase
HDAC, histone deacetylase
PBMCs, peripheral blood mononuclear cells
OVA, ovalbumin
GR, glucocorticoid receptor
LABA, long-acting β_2 agonist
UPS, ubiquitin–proteasome system
siRNA, small interfering RNA

ABSTRACT

Asthma and chronic obstructive pulmonary disease are inflammatory lung disorders responsible for significant morbidity and mortality worldwide. While the importance of allergic responses in asthma is well known, respiratory viral and bacterial infections and pollutants especially cigarette smoke are important factors in the pathogenesis of both diseases. Corticosteroid treatment remains the first preference of treatment in either disease, however these therapies are not always completely effective, and are associated with side effects and steroid resistance. Due to such limitations, development of new treatments represents a major goal for both the pharmaceutical industry and academic researchers. There are now excellent reasons to promote NF-κB signalling intermediates and Rel family proteins as potential therapeutic targets for both asthma and chronic obstructive pulmonary disease. This notion is supported by the fact that much of the underlying inflammation of both diseases independent of stimuli, is mediated at least in part, by NF-κB mediated signalling events in several cell types. Also, a range of inhibitors of NF-κB signalling intermediates are now available, including DNA oligonucleotides and DNA-peptide molecules that act as NF-κB decoy sequences, small molecule inhibitors such as IKK-β inhibitors, and proteasome inhibitors affecting NF-κB signalling, that have either shown promise in animal models or have begun clinical trials in other disorders. This review will focus on the role of NF-κB in both diseases, will discuss its suitability as a target, and will highlight recent key studies that support the potential of NF-κB as a therapeutic target in these two important inflammatory lung diseases.

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doi:10.1016/j.pharmthera.2008.09.003

Contents

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are complex, multifactorial airway diseases associated with significant morbidity and mortality worldwide. Asthma may be caused by allergic responses, and COPD is highly associated with exposure to cigarette smoke (CS), however both diseases may be exacerbated by viral or bacteria infections. In fact, both asthma and COPD are among the top 10 most devastating diseases in the world caused by infectious agents, in terms of loss of life and overall morbidity (Mizgerd, 2006). Despite this importance, both diseases feature distinct clinical phenotypes which are inadequately controlled by inhaled corticosteroid (GC) based therapies, the mainstay therapy for both diseases. The identification of new therapeutic targets therefore remains a research priority for both diseases.

Intense research efforts have described many of the cells and molecules associated with either disease, and while the aetiology of both diseases is multifactorial, both diseases have a significant inflammatory component. Strikingly, many of the pro-inflammatory chemokines, cytokines, adhesion molecules, respiratory mucins, growth and angiogenic factors are induced via the Rel/Nuclear Factor-κB (NF-κB) family of transcription factors. This review will provide a timely summary of the literature regarding the importance of NF-κB in asthma and COPD. We will reflect on the various forms of asthma and COPD, including both stable forms and exacerbations of either disease, and discuss the relative roles of NF-κB in each. We will also argue that asthma and COPD are inflammatory diseases, and that targeting the inflammatory pathways via NF-κB inhibition is a realistic and logical strategy for future therapeutic intervention. We also discuss how drugs targeting NF-κB may have distinct advantages over inhaled GCs at least for some phenotypes of asthma or COPD.

2. Asthma and chronic obstructive pulmonary disease are inflammatory airway diseases

2.1. Overview of asthma

Asthma is defined as variable airway obstruction usually accompanied by airway hyperreactivity (AHR). Defining features of asthma include bronchoconstriction due to contraction or hypertrophy of airway smooth muscle (ASM), and inflammation within the airway. These processes lead to decreased lung function, measured either as changes in peak expiratory flow (PEF) over time, or a decrease in forced expiratory volume (FEV) in 1 s following provocation with histamine. Symptoms often include dyspnoea, wheeze and tightening of the chest. In most cases, the immediate effect on lung function can be reversed by a short acting β_2 agonist. Asthma is now accepted to be a heterogeneous complex disorder (for a review see Wenzel, 2006), and normally presents as a chronic, stable disease with acute exacerbations, often following acute viral or bacterial infection of the lower airway. Exposure to allergens, and the resulting allergic cascade is also a cause of asthma, and this may work in an additive or synergistic manner with exposure to pollution (Spannhake et al., 2002; Chauhan et al., 2003), or acute respiratory infections (Green et al., 2002).

Asthma is associated with early onset in life and affects approximately 20% of the population, with an increased incidence in the developed world (Asher et al., 2006). Asthma may cause severe morbidity, and even mortality due to exacerbation. Asthma is a huge burden on healthcare costs world wide. In the UK, the cost of asthma exacerbations are 3.5 fold higher per patient compared to stable asthma (Hoskins et al., 2000). In raw terms, the annual cost of asthma in terms of GP consultations, emergency room admissions, sick leave, school absenteeism and treatment, is in the order of billions of GBP.

Much is known regarding the cells and molecules contributing to asthma. The allergic cascade has been a focal point of research interest for decades. Upon allergen exposure, mast cells within the airway sensitised with allergen specific IgE are triggered to release a number of pre-formed mediators packaged in their granules. The granules contain histamine, which act on ASM and causes the immediate bronchoconstriction of the airway, observed in asthma. Mast cells synthesise prostaglandins, leukotrienes and kinins which can contribute to bronchoconstriction, and are also a source of the Th2 cytokines IL-4 and IL-13, which serve to increase class switching to IgE in B cells, and augment the production of various Th2 chemokines from epithelial and ASM cells. IL-13 is also highly associated with airway hyperreactivity in animal models of asthma (Leigh et al., 2004; Yang et al., 2004). Mast cells also produce various chemokines attracting inflammatory neutrophils, Th1 and Th2 lymphocytes, and eosinophils into the airway. Th2 lymphocytes are a further source of Th2 cytokines, including IL-5, essential for eosinophil differentiation from bone marrow (for a review, see Renauld, 2001). Recruitment of these various cell types, especially Th2 cells are thought to contribute to the late asthmatic response, (LAR) which features further bronchoconstriction and AHR. In severe asthma, increased ASM proliferation, and fibroblast differentiation may contribute to airway remodelling, causing further long term limitations in lung function via increasing basement membrane thickness, and angiogenesis.

Respiratory viral infections notably human rhinoviruses (RV) respiratory syncytial virus (RSV), coronaviruses and influenza viruses

(Johnston et al., 1993; Johnston et al., 1995; Freymuth et al., 1999; Rakes et al., 1999) are responsible for the majority (>80%) of asthma exacerbations. Atypical bacteria such as *Mycoplasma pneumoniae* and *Chlamydiophila pneumoniae* are also associated with asthma exacerbations (Thumerelle et al., 2003). Both viral and bacterial infection cause upregulation of a plethora of pro-inflammatory molecules in an NF-κB dependent manner, including neutrophil chemokines IL-8/CXCL8 (Johnston et al., 1997; Zhu et al., 1997; Carter et al., 1998; Pizzichini et al., 1998; Cheon et al., 2008), ENA-78/CXCL5 (Donninger et al., 2003) GRO-α/CXCL1 (Edwards et al., 2006a), T cell chemokines IP-10/CXCL10 (Spurrell et al., 2005), Rantes/CCL5 (Thomas et al., 1998; Rudd et al., 2005; Veckman et al., 2005), and eotaxin/CCL10 (Papadopoulos et al., 2001). In addition, various cytokines and growth factors are induced, including IL-6 (Zhu et al., 1996; Dallaire et al., 2001; Oliver et al., 2006; Edwards et al., 2007) GM-CSF (Kim et al., 2000; Funkhouser et al., 2004), IL-11 (Einarsson et al., 1996), fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) (Volonaki et al., 2006). Adhesion molecules (Papi & Johnston, 1999a,b; Murdoch et al., 2002), respiratory mucins including MUC5AC (He et al., 2004; Inoue et al., 2006) and MUC5B (Inoue et al., 2006) are also produced. Macrophages, possibly through recognition of viral and bacterial products including single stranded RNA (ssRNA), double stranded RNA (dsRNA) and lipopolysaccharide (LPS) produce TNF-α (Laza-Stanca et al., 2006; Liu et al., 2008; Oliver et al., 2008), a highly inflammatory cytokine serving to maintain the pro-inflammatory environment within the airway, and is a further inducer of neutrophil and T cell attracting chemokines. Viral induced asthma exacerbations in asthmatic individuals therefore have all the cellular hallmarks one would expect from the production of the above mediators, and commonly feature increased levels of airway eosinophils, neutrophils, and lymphocytes (Fraenkel et al., 1995; Grunberg et al., 1997; Pizzichini et al., 1998; Message et al., 2008). Both experimental human and mouse models of rhinovirus induced asthma exacerbations have recently been developed (Bartlett et al., 2008; Message et al., 2008; Newcomb et al., 2008).

Regarding treatments, GC based therapies have been the most widely used therapy. While very effective in treating stable asthma they are less effective in treating exacerbations (Pauwels et al., 1997; FitzGerald et al., 2004). The allergic response has been an intense focus for therapeutic intervention, culminating in clinical trials using IL-4, IL-5 and IgE as therapeutic targets. Anti-IgE therapy appears partially effective (Bousquet et al., 2004; Holgate et al., 2004), particularly in severe asthma. The outcomes of trials with both soluble IL-4 receptors and humanised anti-IL-5 monoclonal antibodies however have been disappointing (Borish et al., 1999; Leckie et al., 2000; Borish et al., 2001; Kips et al., 2003; O'Byrne et al., 2004). Recent clinical research programs have focused on anti-TNF-α antibodies or soluble TNF-α receptors as a therapy (Howarth et al., 2005; Berry et al., 2006; Erin et al., 2006; Morjaria et al., 2008), highlighting the importance of inflammation as well as Th2 responses in asthma.

2.2. Overview of chronic obstructive pulmonary disease

COPD is defined as a disease state characterized by chronic airflow limitation that is not fully reversible (ERS/ATS COPD Guidelines 2005). The airflow limitation is usually progressive and is associated with an abnormal inflammatory response following a range of different stimuli including CS, pollution, and pulmonary viral or bacterial infection. The clinical characteristics of cough, shortness of breath and sputum production reflect the underlying pathological changes. Mucus hypersecretion and cillary dysfunction lead to collapse of damaged small airways producing airflow limitation, gas trapping and the characteristic obstructive picture on spirometry. While the aetiology of COPD is certainly associated with smoking, the phenotype observed in COPD is variable and complex, and is likely the result of both various genetic and environmental factors.

COPD is associated with an onset later in life, and the incidence is increasing with an ageing population. It affects over 5% adults, is the fourth leading cause of death worldwide and is the only major cause of death that is still rising (Pauwels & Rabe, 2004). It has been predicted from the increasing prevalence that COPD will be the third leading cause of death worldwide by 2020 (Murray & Lopez, 1997).

Stable COPD is punctuated by exacerbations, 50–70% of which are associated with infection by bacteria and viruses (Ball, 1995; Seemungal et al., 2000; Papi et al., 2006). The noncapsulated bacteria *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* are the most frequently isolated bacteria in stable COPD and during exacerbations (Monso et al., 1995). Viruses are detected in at least 40% of COPD exacerbations, and commonly include RV and RSV (Seemungal et al., 2001).

Exacerbations are the major cause of morbidity and mortality in COPD. Exacerbations result in a faster decline in lung function (Donaldson et al., 2002), the number and severity increase with worsening disease (Donaldson et al., 2003), and they are associated with a 7.4% mortality rate in hospitalised patients (Price et al., 2006).

Recently much has been learnt regarding the mechanism by which CS and pulmonary infections contribute to COPD. Stable COPD is generally associated with increased airway CD8+ T lymphocytes, macrophages and neutrophilia (Saetta et al., 1998; Saetta et al., 1999; Qiu et al., 2003; Baraldo et al., 2004), and in some patients, eosinophils (Rutgers et al., 2000). COPD and CS are also highly associated with reactive oxygen, as measured by 8-Isoprostane analysis (Montuschi et al., 2000), reactive oxygen is believed to be central to COPD, by inducing lung damage and hence the initiation of inflammation. During exacerbations, the cellular infiltrate may be more variable, and also demonstrate increased neutrophil chemokines such as ENA-78 and IL-8 (Qiu et al., 2003). COPD exacerbations are also related to an increase in systemic inflammatory molecules, including c-reactive protein and TNF-α (Gan et al., 2004). Recently, a small pilot study has demonstrated that experimental RV challenge is feasible in COPD patients, and induced changes typically experienced during exacerbations of COPD (Mallia et al., 2006).

Both inhaled long acting B_2 agonists (LABAs) and short acting B_2 agonists are used in the symptomatic treatment of COPD. Anticholinergics are also administered, and both B_2 agonist and anticholinergic therapy act mainly by inducing bronchodilation, thereby reducing dyspnoea and may also reduce exacerbation frequency. The use of inhaled GCs in COPD remains controversial, with inhaled GC use recommended for moderate/severe COPD rather than mild COPD. Recently, a large clinical trial has shown improvements in lung function, and exacerbation frequency in individuals receiving combined inhaled GC and LABA treatment versus placebo (Calverley et al., 2007). Oral GC therapy is also recommended for the treatment of COPD exacerbations. An update on current treatment regimes for COPD is provided by the Global Strategy for the Diagnosis Management and Prevention of COPD (Rabe et al., 2007).

3. Nuclear factor-κB signalling and the Rel protein family

NF-κB is a transcription factor expressed in numerous cell types, which plays a key role in the expression of many pro-inflammatory genes, leading to the synthesis of cytokines, adhesion molecules, chemokines, growth factors and enzymes (reviewed in Baldwin, 2001). NF-κB or Rel family members are believed to play a central role in a variety of acute and chronic inflammatory diseases. For this reason the NF-κB signalling pathway has been the focus of extensive research over the last 20 years. NF-κB is activated in response to a number of stimuli, including physical and chemical stress, LPS, dsRNA, ssRNA, T and B cell mitogens and pro-inflammatory cytokines (Rothwarf & Karin, 1999; Karin & Lin, 2002; Li & Verma, 2002; Karin et al., 2004). NF-κB induced gene expression is controlled by a complex series of enzymatic signalling events, at multiple levels. An overview of the NF-κB activation cascade is depicted in Fig. 1.

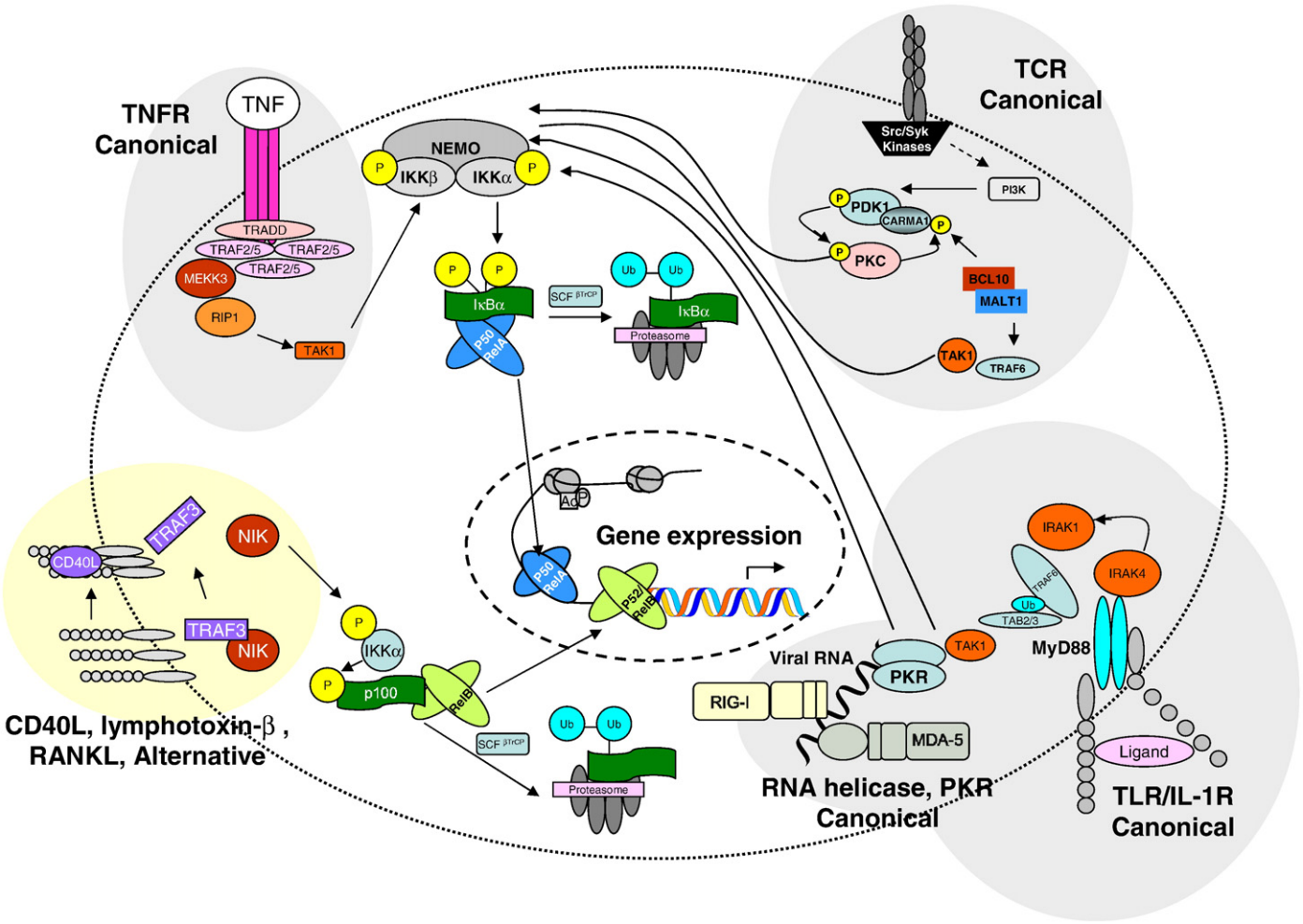

Fig. 1. Signalling pathways leading to NF-κB activation. The canonical pathways include TLR/IL-1 receptors, leading to IRAK activation and IKK-β phosphorylation, intracellular viral receptors including the RNA helicases and PKR which activate IKK-β, the TCR activation pathway, leading to IKK-α IKK-β activation, TNFR activation which signals via TRADD to activate IKK-β, and the alternative pathway induced by CD40–CD40L activation, lymphotoxin-β or RANKL leading to activation of NIK and IKK-α.

Rel family members share an N-terminal Rel homology domain (RHD) which mediates dimerisation, nuclear translocation, and the binding of specific κB sites within promoters of affected genes. The consensus κB site is the decameric sequence 5′-GGGAATTTCC-3′, however extensive variations exist. In mammals there are five known members of the Rel family: p50 (NF-κB1, precursor of which is p105), p65 (Rel A, NF-κB3), p52 (NF-κB2, precursor of which is p100), c-Rel and Rel B. The p50 and p65 subunits are ubiquitously expressed, whereas the other three are generally restricted to specific differentiated cell types (reviewed in Siebenlist et al., 1994). In resting cells, the majority of NF-κB is bound to IκB inhibitory protein which masks the nuclear localisation sequence (NLS) and holds the complex in the cytoplasm. There are a number of different IκB proteins such as IκBα, IκBβ, IκBγ, IκBε and Bcl-3. It is generally believed that the different isoforms are associated with particular Rel protein dimers, bound via their RHD. For example IκBα and IκBβ associate with p65:p50 and p50:c-Rel, whereas IκBε only binds to p65 and c-Rel hetero and homodimers. Upon appropriate stimulation, the IκB protein is phosphorylated and ubiquinated, and subsequent 26S proteasome-mediated degradation. IκB isoform phosphorylation is stimulus specific, for example IκBβ is only phosphorylated by certain stimuli including LPS and IL-1β, whereas IκBα phosphorylation is triggered by most NF-κB activators. This level of control is also thought to impact on the cell type specificity and kinetics of the response, which in turn can influence the duration of transcription.

IκB phosphorylation and activation of Rel proteins can occur via the classical (canonical) or alternative pathway. In the classical pathway, a critical phosphorylation of the IκB protein is performed by the IκB kinase (IKK) complex, which consists of at least three subunits, two catalytic subunits IKK-α/IKK-1 and IKK-β/IKK-2 and a regulatory subunit IKK-γ/NF-κB essential modulator (NEMO) (Scheidereit, 1998; Karin, 1999; Courtois et al., 2001). Of the two catalytic subunits IKK-β is 20 fold more active than IKK-α in phosphorylation of IκB (Mercurio et al., 1997). In the classical pathway, it has been shown that IKK-β, and not IKK-α, is important in NF-κB activation, and the two kinases have distinct rather than overlapping functions (Hu et al., 1999; Li et al., 1999a,1999b; Takeda et al., 1999). The classical pathway includes signalling from TLR/IL-1R family members, intracellular pattern recognition receptors including retinoic acid inducible gene (RIG-I), melanoma differentiation associated factor-5 (MDA-5), and protein kinase R (PKR). Ligation of the T-cell receptor (TCR), and signalling from the TNFR are also examples of the classical pathway (reviewed in (Hacker & Karin, 2006) (Fig. 1).

The alternative pathway is activated by mediators such as lymphotoxin-β, CD40 ligand, and receptor activator of NF-κB ligand (RANKL) (Dejardin et al., 2002; Novack et al., 2003), as shown in Fig. 1. This pathway involves IKK-α phosphorylating and processing of the p52 precursor p100, and nuclear translocation of the heterodimer p52:Rel B (Senftleben et al., 2001). This pathway is believed to play key roles in secondary lymphoid organogenesis and adaptive immunity (reviewed in (Lawrence & Bebien, 2007).

The NF-κB pathway can be further controlled by post translation modifications, including modulating the interaction of Rel proteins with

other components of the transcriptional machinery, and altering their kinetics in and out of the nucleus. The phosphorylation status of NF-κB can influence activation, for example phosphorylation of p65 may enhance transcriptional activation, however phosphorylation of p105 can reduce its processing into p50 (Naumann & Scheidereit, 1994). NF-κB can also associate with other transcriptional proteins such as histone acetyltransferase (HAT) and histone deacetylase (HDAC) (reviewed in Ito et al., 2007). Similarly acetylation of the Rel proteins can increase the time it is located in the nucleus (Chen et al., 2002). Furthermore recent data would suggest that these two modification steps occur in a stepwise manner, phosphorylation of RelA is required prior to and enhances acetylation of Rel A, and improved transcriptional activity (Chen et al., 2005). Hence the NF-κB pathway represents a well studied signal transduction pathway, relevant to many human diseases. The next part of this article will focus on how this knowledge has been applied to generating new therapies for asthma and COPD.

4. Nuclear factor-κB in asthma and chronic obstructive pulmonary disease: evidence from expression levels in diseased tissue, models using gene deficient mice, and in vitro assays

4.1. Asthma

Several lines of evidence indicate enhanced NF-κB pathway activation in asthmatic tissues. Peripheral blood mononuclear cells (PBMCs) of adult uncontrolled, severe and moderate asthmatics have higher levels of NF-κB p65 protein expression, IκB phosphorylation and IKK-β protein levels than normal individuals (Gagliardo et al., 2003). Also in children, NF-κB p65 protein abundance and IκB phosphorylation are also higher in moderate asthmatic PBMCs when compared to normal individuals (La Grutta et al., 2003). Furthermore, when compared to non-asthmatic individuals, nuclear extracts from bronchial biopsies, sputum cells (Hart et al., 1998), and cultured bronchial epithelial cells (Zhao et al., 2001) from stable, untreated asthmatics have greater levels of NF-κB p65 and p50 activation, as measured by gel shift assays and immunofluorescence of nuclear NF-κB p65 protein.

Small animal models of allergic asthma using gene deficient mice highlight the importance of NF-κB in disease pathogenesis. While a wealth of small animal studies have demonstrated the role of NF-κB in inflammation following respiratory viral or bacterial infections (Haeberle et al., 2002; Haeberle et al., 2004; Sadikot et al., 2006; Quinton et al., 2007), NF-κB also appears to be important in the allergic response. In the ovalbumin (OVA) sensitisation and challenge model, total lung extracts from Brown-Norway rats exhibit enhanced NF-κB activity (Lin et al., 2000). In a murine model, bronchial epithelium exhibits robust and rapid NF-κB p65 nuclear translocation and IKK-α/β activity, compared to controls (Poynter et al., 2002). Mice that lack NF-κB p50 have reduced eosinophilic responses to aerolised allergen. This effect was shown to be due to a lack of T cell production of Th2 cytokines, IL-13, IL-4 and the eosinophil growth factor IL-5 (Das et al., 2001), and also the chemokine eotaxin (Yang et al., 1998).

Since, IKK-β plays a crucial role in the classical NF-κB pathway, there has been considerable interest in studying and developing ways to manipulate this kinase. Initial IKK-β gene deletion studies in mice resulted in TNFα-dependent liver degeneration which led to embryonic lethality (Li et al., 1999a,b). This limitation has been overcome through the creation of tissue specific deficient mice, with targeted deletion of IKK-β in Clara epithelial cells in the bronchial tissue (Broide et al., 2005). Upon OVA sensitisation and challenge, airway eosinophils, mucus, peribronchiolar fibrosis, eotaxin and the Th2 T cell chemokine TARC were reduced compared to littermate controls with functional IKK-β. This work demonstrated the importance of NF-κB signalling in both allergic inflammation and mucus production that are relevant to asthma.

There is also an extensive literature concerning NF-κB inhibition in lung cells in vitro, using a range of pharmacological inhibitors, dominant negative kinase mutants (Nasuhara et al., 1999; Li et al., 2003; Catley et al., 2005) and constitutively expressed IκB proteins that sequester NF-κB in the cytoplasm (Thomas et al., 1998; Ciesielski et al., 2002). These studies not only further underscore the importance of NF-κB signalling in the generation of pro-inflammatory cytokines, chemokines and adhesion molecules (Nasuhara et al., 1999; Catley et al., 2004; Birrell et al., 2005a; Catley et al., 2005; Catley et al., 2006; Li et al., 2006; Dajani et al., 2007; Newton et al., 2007), and also as outcomes of both viral (Zhu et al., 1996; Zhu et al., 1997; Kim et al., 2000; Edwards et al., 2006b; Edwards et al., 2007) and bacterial infection (Krull et al., 2006), but provide a highly useful testing ground for research and development into improved therapeutics.

4.2. Chronic obstructive pulmonary disease

As with asthma, much of the research effort in developing treatments has focused on inhibiting the inflammation associated with COPD. Increased markers of NF-κB pathway activity have been demonstrated in the airways of, or samples from, COPD patients, including sputum macrophages (Caramori et al., 2003) during exacerbations of COPD, and also in bronchial biopsies of stable COPD patients (Di Stefano et al., 2002). In rodent models of COPD involving CS exposure (Marwick et al., 2004) and over expression of the Th2 cytokine IL-13 (Chapoval et al., 2007), NF-κB activation has been implicated in disease pathogenesis.

It has been shown that over expression of IKK-β in mouse airway epithelial cells results in an increase in inflammatory mediators and neutrophilic inflammation that is reminiscent of the COPD airway following bacterial challenge (Sadikot et al., 2006). In addition, inhibition of IKK-β in vivo and in vitro reduced TNF-α induced MUC5AC production, one of the major components of respiratory mucus (Lora et al., 2005). Production of another important respiratory mucin, MUC5B has also been shown to be IKK-β dependent, following RV infection in vitro (N. Bartlett, unpublished observations). Hence growing evidence supports a role of NF-κB signalling in COPD pathogenesis.

In vitro systems have also been used to highlight a critical role for IKK-β in the activation of NF-κB (Conron et al., 2002). Transfection of alveolar macrophages with adenovirus constructs expressing defective IKK-β but not NIK proteins inhibited macrophage activation of NF-κB, and expression of TNF-α, IL-8/CXCL8 and IL-6. Monocyte derived macrophages infected in vitro with RV produce TNF-α in a NF-κB dependent manner, which is sensitive to treatment with the IKK-β inhibitor AS206828 (Laza-Stanca et al., 2006).

One possible caveat to NF-κB inhibition in asthma or COPD is the suppression of beneficial host responses. This is most likely in asthma or COPD exacerbations, which have a viral or bacterial aetiology. In asthma, recent data highlight the importance of type I IFN-β and type III IFN-λs as crucial to the host defence against viral infections (Wark et al., 2005; Contoli et al., 2006). As IFN-β and IFN-λ are induced by viruses in an NF-κB dependent manner (Thanos & Maniatis, 1995; Wathelet et al., 1998; Chu et al., 1999; Osterlund et al., 2005), targeting NF-κB in asthma and COPD may not only reduce harmful pro-inflammatory and allergen induced responses, but may also reduce beneficial anti-viral responses. This issue requires clarification in appropriate animal models before targeting NF-κB in viral induced asthma exacerbations can be applied in human studies. A list of cytokines, chemokines and other molecules regulated in an NF-κB dependent manner associated with asthma or COPD is provided in Table 1.

5. Available inhibitors for in vivo inhibition of nuclear factor-κB

5.1. Corticosteroid based treatments

Inhaled or oral GCs remain the most effective treatment for asthma and COPD. The glucocorticoid receptor (GR) is a cytoplasmic steroid hormone receptor that undergoes a conformational change and dimerisation upon ligation with GC, and rapid nuclear translocation.

Table 1
A comprehensive list of known downstream targets of the transcription factor NF-κB relevant to asthma and/or COPD

Downstream targets of NF-κB inhibition relevant to asthma and COPD		
Protein/Gene	Function	Reference
Cytokines		
TNF-α	Inflammatory cytokine	(Laza-Stanca et al., 2006)
IL-1β	Inflammatory cytokine	(Haddad, 2002)
IL-6	Lymphocyte and macrophage maturation	(Zhu et al., 1996)
GM-CSF	Neutrophil generation from bone marrow	(Funkhouser et al., 2004)
IL-5	Th2 cytokine	(Mori et al., 1997)
IL-4	Th2 cytokine	(Das et al., 2001)
IL-13	Th2 cytokine	(Das et al., 2001)
Chemokines		
IL-8/CXCL8	Neutrophil chemokine	(Zhu et al., 1997)
ENA-78/CXCL5	Neutrophil chemokine	(Sachse et al., 2006)
NAP-2/CXCL4	Neutrophil chemokine	(Catley et al., 2006)
GRO-α/CXCL1	Neutrophil chemokine	(Issa et al., 2006)
GRO-γ/CXCL3	Neutrophil chemokine	(Bezzerri et al., in press)
TARC/CCL17	Th2 cell chemokine	(Berin et al., 2001)
MIP-3α/CCL20	T cell and immature DC chemokine	(Matsukura et al., 2006)
Eotaxin/CCL10	Eosinophil chemokine	(Yang et al., 1998)
Rantes/CCL5	Th1 cell chemokine	(Thomas et al., 1998)
IP-10/CXCL10	Th1 cell chemokine	(Spurrell et al., 2005)
MCP-1	Monocyte chemokine	(Catley et al., 2006)
Mucins		
MUC5AC	Respiratory mucin	(Kraft et al., 2008)
MUC5B	Respiratory mucin	(Inoue et al., 2006)
MUC7	Respiratory mucin	(Li & Bobek, 2006)
Receptors		
ICAM-1	Leukocyte adhesion	(Papi & Johnston, 1999b)
VCAM-1	Leukocyte adhesion	(Papi & Johnston, 1999a)
CD23	Low affinity receptor for IgE	(Debnath et al., 2007)
CD21	CD23 co-receptor	(Debnath et al., 2007)
IgE	Allergen binding on mast cells and basophils	(Oomizu et al., 2006)
Enzymes		
COX-2	Coverts arachidonic acid to prostaglandins	(Steer et al., 2003)
iNOS	Produces nitric oxide	(Li et al., 2002)
MMP9	Protease associated with remodelling of the extracellular matrix and cell migration	(Rhee et al., 2007)

In vitro studies show that GR acts via a range of different mechanisms, including prevention of NF-κB interacting with it's cis-acting site, with other transcription factors, or structural proteins required for transcription through the process broadly known as trans-repression (Tuckermann et al., 1999; Tao et al., 2001). GR activation can lead to expression of phosphatases that prevent inflammatory kinases from signalling (Issa et al., 2007), or via recruiting HDACs that prevent histone acetylation, disassociation of chromatin, and therefore NF-κB binding at a given promoter (Ito et al., 2000; Ito et al., 2001). How GCs impact on NF-κB signalling, is shown in Fig. 2.

GCs are often used in conjunction with other therapies, such as LABAs or leukotriene receptor antagonists. GC based therapies are most effective in the treatment of stable or allergic asthma, are less effective in COPD, and even less so for exacerbations of either disease (Pauwels et al., 1997; Calverley et al., 2003). Despite the continued reliance on GC based therapies in asthma and COPD, a major research objective is to find better treatments for exacerbations of asthma and COPD.

Several studies have examined the efficacy of GC based therapies in reducing NF-κB activity from ex vivo material, with mixed results. In patients with stable asthma undergoing treatment with budesonide, budesonide increased GR–DNA binding in bronchial biopsies and reduced NF-κB-DNA binding (Hancox et al., 1999). In support, Wilson et al. have demonstrated a reduction in activated NF-κB staining after treatment with budesonide or the LABA formoterol (Wilson et al., 2001). Bronchial biopsies from budesonide treated individuals also had significantly less IL-8, TNF-α and GM-CSF, however no difference was observed for formoterol treated samples. In contrast, Hart et al., studying NF-κB–DNA binding in stable asthmatic bronchial biopsies and alveolar macrophages after treatment with fluticasone propionate, showed no decrease in NF-κB activity compared to a placebo administered control group. Fluticasone was effective however at reducing BAL eosinophils and improved lung function (Hart et al., 2000). Together, the data suggest that in lung tissue, GCs are incompletely effective in blocking NF-κB activity and that the anti-inflammatory activity of GCs may act via other mechanisms.

The effectiveness of GCs in controlling NF-κB mediated allergic responses from lymphocytes has also been examined. T cell clones from stable asthmatics synthesised IL-5 upon stimulation with anti-TCR antibodies or IL-2 treatment (Mori et al., 1997). Dexamethasone effectively reduced IL-5 synthesis in both models. The authors also demonstrated that the targets for GC action were likely to be NF-κB and AP-1. Fluticasone and salmeterol also caused decreased phospho-IκB levels in asthmatic T cells (Pace et al., 2004), suggesting that IκB may also be a target for GC action.

There are currently very few studies examining the effects of GC based therapies on NF-κB expression following asthma or COPD exacerbations. While clearly less effective than in stable disease, the lack of efficacy in exacerbations remains poorly understood. One theory is that as most exacerbations have viral and bacterial aetiologies, this may involve distinct mechanisms, involving different cells and or molecules than in stable forms of either disease. In vitro, in normal tissue, GC based therapies reduce inflammatory mediators induced by viral and non-viral stimuli (IL-1β) with about the same efficacy (Edwards et al., 2006a; Edwards et al., 2007), arguing against the above. This is also supported by the fact that much of the underlying inflammation in both stable, non-viral and viral or bacterial induced exacerbations involves NF-κB. More research, particularly in asthmatic and COPD tissue, and the testing of known NF-κB inhibitors in models of asthma or COPD exacerbation are required to carefully scrutinize these mechanisms.

5.2. Decoy oligonucleotides

As the cis-acting sites within various promoters have a conserved motif, NF-κB decoy oligonucleotides which bind activated Rel proteins and prevent them from binding to their nuclear targets represent potential therapeutic agents. These oligonucleotides, or their DNA-peptide orthologs, have shown proof of concept in vitro (Tomita et al., 1998; Mischiati et al., 1999; Romanelli et al., 2001) and have been studied in various animal models including sepsis (Matsuda et al., 2004) and asthma (Desmet et al., 2004). Furthermore, decoy oligonucleotides to the cell cycle regulator E2F have been trialled ex vivo in clinical trials for the treatment of vein graft rejection (Mann et al., 1999; Alexander et al., 2005).

Using the OVA challenged and sensitised mouse model of asthma, Desmet et al. assessed the efficacy of NF-κB decoy oligonucleotides during ovalbumin induced allergic airway inflammation (Desmet et al., 2004). Initial experiments demonstrated that the target cell population within the lung consisted of DCs, T cells, macrophages and granulocytes, located with the bronchial and perivascular areas of the lung. Interestingly, structural cells, including bronchial epithelial cells were not transfected in this study. At 24 h post treatment, mice treated with NF-κB decoy oligonucleotides exhibited lower BAL eosinophils, neutrophils, lymphocytes and macrophages compared to untreated, ovalbumin challenged mice or mice treated with a scrambled, non-specific oligonucleotide. Differences were also observed in BAL cytokines, with IL-13, IL-5, IFN-γ, and eotaxin being lower in mice challenged with ovalbumin and treated with the NF-κB decoy oligonucleotide. Treated animals also exhibited less AHR than control groups, strongly suggesting that inhibiting NF-κB can directly affect lung function in this model. Finally PAS staining of lung sections revealed lower levels of mucin protein in OVA challenged NF-κB decoy

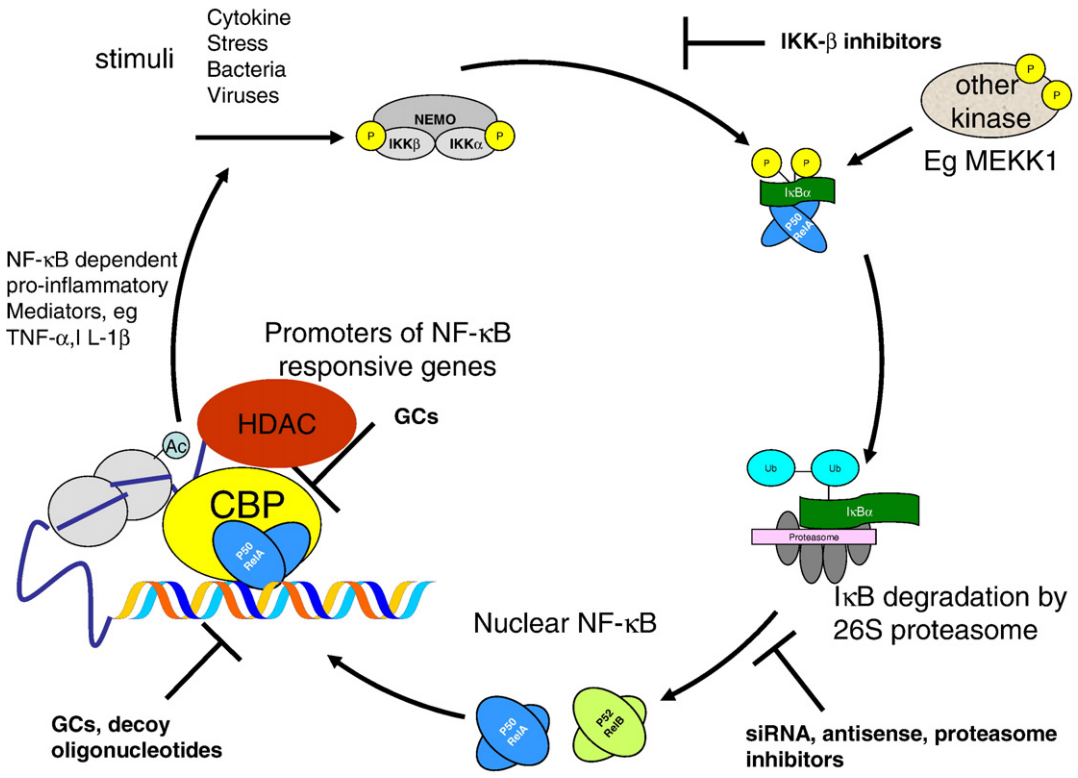

Fig. 2. Inhibitors of the NF-κB pathway. Small molecule or peptide inhibitors of IKK-β prevent IKK-β phosphorylation, proteasome inhibitors prevent IκB processing, antisense and siRNA act on NF-κB mRNA and limit subsequent protein expression, decoy oligonucleotides compete for the κB sites within promoters of affected genes preventing their transcriptional activation. GCs may act by binding activated NF-κB family members and preventing their association with κB sites (trans-repression) or by recruiting HDACs, and preventing chromatin disassociation, and hence assess to κB sites within affected genes.

oligonucleotide treated animals compared to control animals. IL-4, and IgE levels were not affected, suggesting that not all components of the allergic cascade can be affected using this strategy.

5.3. Small molecule inhibitors of I-κB kinase-β

As described above, there is now substantial data to suggest that inhibition of IKK-β could have beneficial disease modifying properties. Targeting IKK-β represents a growing area of interest for academic researchers and industry alike, as a plethora of small molecule inhibitors have recently become available. As yet, there has been no clinical trials using IKK-β inhibitors in asthma or COPD, however several inhibitors have been used in phase I and II clinical trials in other disorders, notably cancer (reviewed in Karin et al., 2004). These compounds are widely used in research, particularly in small animal models. Currently there are at least eight small molecule inhibitors of IKK-β available, with efficacies in cell based in vitro assays in the low micromolar range. A list of these inhibitors is presented in Table 2.

IKK-β inhibitors have been rigorously tested in cell based assay systems and show reliable anti-inflammatory activity using a range of stimuli (Birrell et al., 2005a; Ziegelbauer et al., 2005; Catley et al., 2006; Issa et al., 2006; Newton et al., 2007). It would appear that IKK-β inhibitors may possess a more comprehensive anti-inflammatory profile to that of GCs, for instance IL-1β induced G-CSF release from primary ASM is virtually steroid resistant, whereas it is completely blocked by two structurally different IKK-β inhibitors (Birrell et al., 2005a). This is potentially very exciting, because the inflammation in COPD patients, and a sub-population of asthmatics are reportedly resistant to GC treatment. Peroxynitrite, and nitration of HDACs, caused by reactive oxygen produced by CS, have been implicated in

Table 2
Available small molecule IKK-β inhibitors

Compound	IC50, assay	Reference
TPCA-1 2-[(Aminocarbonyl)amino]-5-[4-fluorophenyl]-3-thiophenecarboxamide	0.17–0.32 μM, pro-inflammatory cytokine expression in monocytes	(Podolin et al., 2005)
PS-1145 N-(6-chloro-9H-beta-carbolin-8-ly) nicotinamide	Data not provided	(Hideshima et al., 2002)
ML120B N-(6-chloro-7-methoxy-9H-beta-carbolin-8-yl)-2-methyl-nicotinamide	3.3 μM TNF-α expression in PBMCs	(Wen et al., 2006)
SC-514 5-(Thien-3-yl)-3-aminothiophene-2-carboxamide	8–20 μM pro-inflammatory cytokine expression in synovial fibroblasts	(Kishore et al., 2003)
IMD-0354 N-[3,5-bis-trifluoromethyl-phenyl]-5-chloro-2-hydroxy-benzamide	Data not provided	(Onai et al., 2004)
BMS-345541 4(2′-aminoethyl)amino-1,8-dimethylimidazo(1,2-aquinoxaline)	4 μM IκB phosphorylation in THP-1 cells	(Burke et al., 2003)
BAY 11-7085 3-[4-*t*-butylphenyl)-sulfonyl]-2-propenenitrile	10 μM, adhesion molecule expression in HuVEC cells	(Pierce et al., 1997)
AS602868	1–2 μM, NF-κB activation in Jurkat T cells	(Frelin et al., 2003)

causing steroid resistance in COPD (Barnes et al., 2004; Ito et al., 2005). Also, a sub population of asthmatics, are believed to have reduced GR levels, GR translocation and recruitment of HDAC, reducing GC efficacy (Adcock et al., 1995a; Adcock et al., 1995b; Matthews et al., 2004). Hence a distinct advantage of IKK-β inhibition is to bypass these problems associated with GC based therapies. Furthermore, a recent publication has suggested that IKK-β inhibition may have additional anti-inflammatory activity which is separate to the impact on NF-κB signalling, as shown using the IKK-β inhibitor TCPA-1, a structural different IKK-α/β inhibitor, and dominant negative IKK-β (Tudhope et al., 2007). The mechanism however is currently unknown. Also, a cell permeable peptide which specifically disrupts the IKK-β/NEMO interaction (May et al., 2000), has been used in vitro to block NF-κB signalling in an IKK-β dependent manner (Dai et al., 2004). Such small peptide inhibitors are also valuable tools, to highlight mechanisms in vitro and perhaps also as therapeutics in vivo.

In small animal models of asthma, IKK-β inhibitors have been shown to impact on a number of relevant end points. These include inflammatory cytokines, mucus, LAR, AHR and fibrosis (Birrell et al., 2005a; Ziegelbauer et al., 2005; Chapoval et al., 2007). For example, the selective IKK-β inhibitor TCPA-1 reduced TNF-α and IL-1β, eotaxin, and Th2 cytokines IL-4, IL-5 and IL-13 induced by OVA in a rat model of allergen sensitisation and challenge. Recruitment of neutrophils and eosinophils into the airway and the late response to allergen challenge were also markedly reduced following TCPA-1 administration (Birrell et al., 2005a). Importantly, these results highlight the ability of TCPA-1 to reduce both Th2 induced responses and general inflammation following allergen challenge.

As yet, less has been reported on the impact of IKK-β inhibitors in similar models of COPD. IKK-β inhibition has been effective in LPS models of COPD (Ziegelbauer et al., 2005; Birrell et al., 2006). Similarly, IL-13 induced inflammation in the mouse is reduced with an IKK-β inhibitor (Chapoval et al., 2007). One might question the clinical relevance of these animal models; for example unlike the inflammation in clinical COPD, the LPS induced inflammation is modulated by GC treatment. Another rodent model of COPD utilising elastase as a stimulus, which is known to be steroid resistant has also recently been shown to also be resistant to an IKK-β inhibitor (Birrell et al., 2006). This result might be expected, as the inflammation observed in this model appears to occur without any obvious signs of NF-κB pathway involvement (Birrell et al., 2005b). Again one may question the clinical relevance of this model, especially considering the apparent increase in the NF-κB pathway activity in patients with COPD (Caramori et al., 2003). However, regardless of whether this model represents human COPD, it appears to demonstrate NF-κB-independent, GC resistant inflammation which could be very interesting to explore. As yet there are no reports on the impact of an IKK-β inhibitor in the more accepted CS driven animal model of COPD. These studies are crucial for the further validation of IKK-β inhibitors in COPD.

5.4. Proteasome inhibitors

The ubiquitin–proteasome system (UPS) regulates protein turnover in eukaryotic cells and is central to the regulation of NF-κB activity. Protein degradation through the UPS involves ubiquitin conjugation to proteins destined for destruction and is mediated by the enzymes E1, E2 and E3. Polyubiquinated proteins are recognised by the proteasome which de-ubiquinates, unfolds and destroys the target protein (reviewed in Nalepa et al., 2006). The 26S proteasome is an ATP-dependent proteolytic complex consisting of a proteolytic core particle, the 20S proteosome, capped on each end by two regulatory complexes. The 20S proteasome contains 3 pairs of active sites with distinct specificities termed chymotrypsin-like, trypsin-like and caspase-like (reviewed in Gilmore and Herscovitch, 2006). There are several classes of reversible and irreversible proteasome inhibitors all of which target the 20S proteasome, some of which have been utilised in asthma or COPD research. Several studies have reported modulation of eosinophil function by blocking NF-κB signalling using proteasome inhibitors. Eosinophils secrete a variety of potentially damaging mediators in response to pro-inflammatory- and microbial stimuli in an NF-κB dependent manner (Rankin et al., 2000). The activity of the lactocystin-derivative proteasome inhibitor PS-519 was examined in a rat model of OVA induced pulmonary eosinophilia. Intratracheal dosing of PS-519 before and after allergen exposure significantly reduced the number of eosinophils in sensitised lungs. Furthermore, a low dose of PS-519 was similarly effective at decreasing eosinophilic inflammation in the airways when used in combination with the GC budesonide, demonstrating the potential for combination treatment regimens with GCs (Elliott et al., 1999).

MG-132 is a member of the peptide aldehyde class of proteasome inhibitors and blocks the chymotrypsin-like activity of the proteasome complex (Palombella et al., 1994). Several studies have demonstrated that MG-132 mediated NF-κB inhibition modulates eosinophil activity and reduces allergic inflammation. TNF-α induced IL-8 by purified human eosinophils was blocked by pre-treatment with MG-132. Additionally, inhibition of NF-κB signalling by MG-132 enabled TNF-α to induce eosinophil apoptosis rather than induce IL-8 production (Fujihara et al., 2002). The expression of cell adhesion molecules such as the β_2 integrin CD18 are critical to eosinophil homing to sites of inflammation, and can be induced by TNF-α or co-culture with bronchial epithelial cells (Teixeira et al., 1994). TNF-α also initiates ICAM-1 expression on bronchial epithelial cells via NF-κB activation (Chen et al., 2001). MG-132 treatment reduced expression of CD18 on eosinophils and ICAM-1 on BEAS-2B cells following co-culture, irrespective of the presence of TNF-α, highlighting a role for bronchial epithelial cells in NF-κB mediated upregulation of cell adhesion molecules on eosinophils (Wong et al., 2006).

The potential to modulate airway chemotactic activity and reduce T cell recruitment via inhibition of the proteasome has also been investigated. Bronchial biopsies from atopic asthmatics treated with the proteosomal inhibitor CBz-Ile-Glu(OtBu)-Ala-leucinal exhibited both reduced T cell chemotactic activity and production of IL-16 (Hidi et al., 2000). TLR agonists are potent activators of the classical NF-κB pathway. Inhibition of the proteasome by MG-132 has also revealed a role for NF-κB and TLR3/4 stimulation in tracheal contraction. One study demonstrated that the expression of TLR2, TLR3 and TLR4 in the smooth muscle layer of mouse trachea increased contractile response to bradykinin following stimulation with TLR agonists LPS and polyIC. This response was associated with nuclear translocation of p65 and up-regulation of kinin B1 and B2 receptor mRNA and could be inhibited by MG-132 (Bachar et al., 2004).

While the role of TLR signalling and NF-κB activation in asthma has been well studied, much less is known about CS induced, TLR mediated inflammation in COPD. Stimulation of macrophages via TLR4 leads to NF-κB activation and production of IL-8 (Brightbill et al., 1999). To assess the role of CS and induction of NF-κB regulated pro-inflammatory gene expression, human monocyte-derived macrophages were treated with CS in vitro and increased IL-8 production was observed. This response was dependent on TLR4 activation and was associated with phosphorylation of IRAK and activation of NF-κB. MG-132 mediated proteasomal inhibition prevented CS induced IκBα degradation (Karimi et al., 2006). The peptide boronic acids, also known as dipeptidyl boronates, originally used as inhibitors of serine proteases, comprise another class of protease inhibitors blocking the chymotrypsin-like site in the 20S subunit core. While these have shown efficacy against several tumours, there is little data describing their use in treating airway inflammation (reviewed in Gilmore & Herscovitch, 2006). Large clinical trials using proteasome inhibitors also highlight potential side effects associated with proteasome inhibitor use. A clinical trial utilising Bortezomib, a boronic acid dipeptide, induced several side effects including thrombocytopenia, fatigue, peripheral neuropathy and neutropenia (Richardson et al.,

2003). It is thought that the side effects could be the result of the expression of many genes and cellular processes influenced by proteasome inhibition, and this concept requires consideration before being applied to studies in asthma and COPD.

5.5. Antisense and small interfering ribonucleic acid

While decoy oligonucleotides competitively bind free NF-κB dimers thus preventing their interaction with cis-acting sites within promoter regions, antisense and small interfering RNA (siRNA) are nucleic acid based agents that target a specific mRNA such as NF-κB and reduce the abundance of the corresponding protein. Antisense technology uses stablilised phosphothionate oligonucleotides to bind to complementary mRNA, thus blocking translation. OVA sensitised mice injected intravenously twice with a p65 antisense oligonucleotide show a significant reduction in the level of p65 protein and NF-κB activation in the lung. The reduction of p65 protein was associated with reduced airway inflammation cell recruitment, AHR, and both pro-inflammatory and Th2 cytokine production in BAL and OVA specific IgE in serum (Choi et al., 2004).

The mRNA of a target protein can also be reduced using siRNA technology, via the process of RNA interference (for a review see Shrivastava & Srivastava, 2008). Once delivered, siRNA transiently silences gene expression by directing sequence-specific degradation of a target mRNA by an endogeneous RNA-induced silencing complex (Elbashir et al., 2001). The majority of reports to date describing the use of siRNA to treat lung disease in vivo have targeted respiratory virus infections (Bitko et al., 2005), demonstrating the general usefulness of delivering siRNA to the airway in vivo. Also, studies using cultured airway epithelial cells have demonstrated the utility of siRNA targeting p65 in reducing TNF-α induced pro-inflammatory cytokine production. Primary epithelial cells transfected with p65-targeting siRNA produced less IL-6 and IL-8 in response to TNF-α, however this depended on the cells being undifferentiated at the time of transfection (Platz et al., 2005). An alternative to using transfection to deliver siRNA to the lungs, is to use a viral delivery system. Another study has employed a recombinant adeno-associated virus expressing a p65-targeting siRNA. Using a similar cellular model to the previous study, expression of siRNA against p65 suppressed secretion of IL-8 by TNF-α-stimulated BEAS-2B cells (Pinkenburg et al., 2004). While the application of this technology in vitro or in vivo is relatively recent, there is growing interest in this field, and as the methodology associated with both the design and delivery of siRNA expands, this approach is certain to broaden the range of therapeutic options available for asthma and COPD.

6. Summary and concluding remarks

The NF-κB pathway is central to the pathogenesis of both asthma and COPD. A large body of evidence has convincingly demonstrated a clear role for NF-κB transcription factors and their signalling kinases in both the stable and exacerbation forms of either disease. These data are the sum of several lines of evidence, and are apparent in investigations using diseased tissue, animal models of disease, and in vitro culture cell systems. While the role of the NF-κB pathway is clear, and methodologies to inhibit NF-κB are encouraging in animal models, which molecules to specifically target and appropriate methods of their inhibition in human studies are less so. There is good evidence for small molecule inhibition methodologies, and also for the use of oligonucleotide based methodologies, including decoy oligonucleotides and siRNA. Further, carefully controlled studies in reliable animal models must be performed to address this complex issue before any form of inhibition can be tested in human studies. Which NF-κB signalling molecule represents the best therapeutic target is again a perplexing question. While knowledge of each NF-κB signalling molecule is expanding, researchers need to respect many variables, including cell type specificity, and which aspect of pathogenesis to inhibit (allergic versus non allergic inflammation, secreted cytokine/chemokine or non-secreted enzyme, adhesion molecule). Another important point worth considering is which end points are most important, especially in animal models. While inhibiting NF-κB clearly affects a range of different end points, including allergic and non-allergic inflammation, and AHR, there does exist some heterogeneity between different models. This is a truly salient point, differential targeting of the NF-κB pathway in different cell types may alter various end points and disease outcome in these animal models. It is likely that each outcome may be model dependent; hence the results of different models need to be interpreted with caution. The above points need to be thoroughly considered in future studies if NF-κB is going to be a serious contender for therapeutic intervention in human disease.

The wealth of literature focusing on NF-κB in asthma and COPD has also teased out several controversies regarding the suitability and or role of NF-κB in asthma and COPD. Firstly, there is the consideration of the role of NF-κB to beneficial host responses to infectious agents. Secondly, there is the conundrum of why known NF-κB inhibitors such as GCs work poorly in controlling exacerbations of asthma and COPD. While the answers are clearly complex, a better understanding, and better defining of each disease in human systems will be important in future studies. Also, particularly for COPD, and exacerbations of both diseases, the design of useful animal models with clinically useful end points is also required. The potential of targeting the NF-κB pathway in asthma and COPD is therefore an excellent example in the study of clinical pharmacology. As the technology and therapeutic agents become increasingly available, so does the opportunity to trial new treatments, and learn more about the mechanisms behind these important diseases.

Acknowledgments

MR Edwards is supported by a Fellowship from Asthma UK and grants from the British Lung Foundation. We thank Annie Sykes for critical reading of the manuscript.

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