

## RESEARCH PAPER

# Potentialiation of NF- $\kappa$ B-dependent transcription and inflammatory mediator release by histamine in human airway epithelial cells

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**Background and purpose:** In asthma, histamine contributes to bronchoconstriction, vasodilatation and oedema, and is associated with the late phase response. The current study investigates possible inflammatory effects of histamine acting on nuclear factor  $\kappa$ B (NF- $\kappa$ B)-dependent transcription and cytokine release.

**Experimental approach:** Using BEAS-2B bronchial epithelial cells, NF- $\kappa$ B-dependent transcription and both release and mRNA expression of IL-6 and IL-8 were examined by reporter assay, ELISA and quantitative RT-PCR. Histamine receptors were detected using qualitative RT-PCR and function examined using selective agonists and antagonists.

**Key results:** Addition of histamine to TNF $\alpha$ -stimulated BEAS-2B cells maximally potentiated NF- $\kappa$ B-dependent transcription 1.8 fold, whereas IL-6 and IL-8 protein release were enhanced 7.3- and 2.7-fold respectively. These responses were, in part, NF- $\kappa$ B-dependent and were associated with 2.6- and 1.7-fold enhancements of IL-6 and IL-8 mRNA expression. The H<sub>1</sub> receptor antagonist, mepyramine, caused a rightward shift in the concentration-response curves of TNF $\alpha$ -induced NF- $\kappa$ B-dependent transcription ( $pA_2=9.91$ ) and release of IL-6 ( $pA_2=8.78$ ) and IL-8 ( $pA_2=8.99$ ). Antagonists of histamine H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub> receptors were without effect. Similarly, H<sub>3</sub> and H<sub>4</sub> receptor agonists did not affect TNF $\alpha$ -induced NF- $\kappa$ B-dependent transcription, or IL-6 and IL-8 release at concentrations below 10  $\mu$ M. The anti-inflammatory glucocorticoid, dexamethasone, inhibited the histamine enhanced NF- $\kappa$ B-dependent transcription and IL-6 and IL-8 release.

**Conclusions and implications:** Potentiation of NF- $\kappa$ B-dependent transcription and inflammatory cytokine release by histamine predominantly involves receptors of the H<sub>1</sub> receptor subtype. These data support an anti-inflammatory role for H<sub>1</sub> receptor antagonists by preventing the transcription and release of pro-inflammatory cytokines.

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**Keywords:** histamine; NF- $\kappa$ B; inflammation; IL-6; IL-8; mepyramine; glucocorticoid; BEAS-2B epithelial cells

**Abbreviations:** CRE, cyclic AMP response element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; I $\kappa$ B, inhibitor of kappa B; MOI, multiplicity of infection; NF- $\kappa$ B, nuclear factor kappa B; PKA, protein kinase A; TNF $\alpha$ , tumour necrosis factor  $\alpha$

## Introduction

Chronic inflammation is a hallmark of airway diseases, including asthma, chronic obstructive pulmonary disease and allergic rhinitis (Chung, 2006; Jeffery and Haahnela, 2006). The production of histamine (2-[4-imidazole]-ethylamine) through activation of histidine decarboxylase in mast cells and basophils and, to a lesser extent, in neurons and lymphocytes, plays a major role in the establishment of the

inflamed state (Jutel *et al.*, 2005). In 1911, histamine was shown to have smooth muscle-stimulating and vasodepressor properties, which mimicked symptoms observed during anaphylaxis (Dale and Laidlaw, 1911). Since this time, histamine has been found to exert its effects via four different G-protein-coupled receptors, termed H<sub>1</sub>–H<sub>4</sub> (Akdiss and Simons, 2006; Alexander *et al.*, 2006). These receptors can couple to different G-proteins and activate divergent signalling pathways. For example, the H<sub>1</sub> histamine receptor couples via G<sub>q/11</sub> to phospholipase C and promotes the hydrolysis of phosphatidylinositol(4,5)bisphosphate into diacylglycerol and inositol(1,4,5)trisphosphate (Tilly *et al.*, 1990; Li *et al.*, 1995). This stimulates calcium mobilization and results in the activation of calcium- and diacylglycerol-

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dependent signalling molecules such as protein kinase C (Liu and Heckman, 1998). In contrast, the histamine H<sub>2</sub> receptor is linked to G<sub>s</sub> and couples to the adenylyl cyclase–cyclic AMP pathway to activate protein kinase A (Flamand *et al.*, 2004). Both the H<sub>3</sub> and H<sub>4</sub> receptors have been shown to be linked to G<sub>i/o</sub> and may inhibit adenylyl cyclase as well as activating mitogen-activated protein kinases (Lovenberg *et al.*, 1999; Morse *et al.*, 2001; Giovannini *et al.*, 2003; Lim *et al.*, 2005).

The transcription factor nuclear factor kappa B (NF-κB) promotes the transcription of over 150 genes, including cytokines, chemokines, adhesion molecules and inflammatory enzymes such as cyclooxygenase-2 (Barnes and Karin, 1997; Pahl, 1999). NF-κB typically exists as a heterodimer of two proteins, p65 (relA) and p50, which, in resting cells, is held in the cytoplasm through its association with one of the proteins from the inhibitor of κB (IκB) family (Karin and Ben-Neriah, 2000). Pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα) or interleukin (IL)-1β, activate the IκB kinase complex, which then phosphorylates the IκB protein, usually IκBα, to target it for ubiquitination and degradation by the 26S proteasome (Karin and Ben-Neriah, 2000). This reveals a nuclear localization signal allowing NF-κB to translocate into the nucleus where it binds to κB sites in the promoters of NF-κB-dependent genes to upregulate transcription (Barnes and Karin, 1997).

The airways epithelium extends from the nose to the terminal bronchioles and acts as the interface between the inhaled air and the respiratory system (Davies and Holgate, 2002). These cells are a point of first contact for pro-inflammatory insults, including airborne allergens, pollutants and pathogens that may exacerbate inflammatory airway diseases (Davies and Holgate, 2002). Importantly, epithelial cells contribute to disease exacerbations by producing pro-inflammatory mediators, cytokines, chemokines and expressing adhesion molecules (Mills *et al.*, 1999). The epithelial layer is also targeted by inhaled therapies such as glucocorticoids, making this cell type a critical site of therapeutic action.

In addition to inducing bronchoconstriction and oedema, histamine is also implicated in the induction of inflammatory cytokines, chemokines and other pro-inflammatory factors (Marone *et al.*, 2003). In the present study, histamine, acting primarily via the H<sub>1</sub> receptor on BEAS-2B bronchial epithelial cells, potentiated TNFα-induced NF-κB-dependent transcription and the release of IL-6 and IL-8.

## Materials and methods

### *Cell culture, cytokines and drugs*

BEAS-2B cells were originally isolated from normal human bronchial epithelial cells obtained from a non-cancerous individual and which had been infected with an adenovirus 12-SV40 virus hybrid (Ad12SV40) (Reddel *et al.*, 1988). An individual colony (BEAS-2B) with unlimited proliferative potential was obtained, which did not show evidence of viral production and which retained the morphological characteristics of bronchial epithelial cells. BEAS-2B cells were grown to confluence in 6 or 24-well plates using Dulbecco's

modified Eagle's medium/F12 medium (Invitrogen, Burlington, ON, Canada) in 10% fetal calf serum, as described previously (Catley *et al.*, 2004). Cells were cultured overnight in serum-free media before changing to fresh serum-free media containing drugs and stimuli.

### *Adenovirus infection*

BEAS-2B cells were either infected with empty (null) Ad5 expression vector or a vector expressing an IκBα protein with an N-terminal deletion (IκBαΔN) (Krappmann *et al.*, 1996). This deletion causes the IκBα to be a constitutive inhibitor of NF-κB. Cells were infected with Ad5-IκBαΔN and null adenoviruses at a multiplicity of infection (MOI) of 30, which results in infection of >95% of cells, as described previously (Meja *et al.*, 2004).

### *NF-κB reporter cells and luciferase assay*

The NF-κB-dependent reporter pGL3.neo.TATA.3κBu was based on the parent vector pGL3.neo.TATA, which contains a neomycin-resistance gene and a modified minimal β-globin promoter driving luciferase (Catley *et al.*, 2004). This was digested at the *Sma*I site, upstream of the minimal β-globin promoter, and a double-stranded oligonucleotide (sense strand: 5'-AGGGGATTCCTAGGGGATTCCTAGGGGATTCCT-3'), containing three copies of the upstream NF-κB site (underlined) derived from the human cyclooxygenase-2 promoter (Newton *et al.*, 1997), was inserted to produce pGL3.neo.TATA.3κBu (referred to as 3κBu-luc). A mutated version (3κBu(mut)-luc) of the reporter was generated as above, but using modified oligonucleotides (sense strand: 5'-AGGccATTCCTAGGccATTCCCTAGGccATTCCCT-3') (mutated bases in lower case), which do not bind NF-κB (Newton *et al.*, 1997). In preliminary experiments, neither the mutated construct nor the parent construct, pGL3.neo.TATA, was responsive to TNFα (data not shown). This indicates that TNFα responsiveness of the 3κBu-luc construct is due to the NF-κB-binding sites. Stably transfected BEAS-2B reporter cells were generated by transient transfection of 8 μg of each plasmid into pre-confluent BEAS-2B cells in T-162 flasks. Cells were cultured in the presence of 0.1 mg ml<sup>-1</sup> G-418 until foci of resistant cells appeared. These were harvested to create a heterogeneous population, randomized for integration site. The lines were expanded for generation of stocks and experimental procedures. Stably transfected BEAS-2B cells with a cyclic AMP response element (CRE)-dependent luciferase reporter that contains six tandem CRE motifs upstream of a β-globin promoter driving a luciferase gene were grown as described previously (Meja *et al.*, 2004). Confluent BEAS-2B reporter cells in 24-well plates were changed to serum-free medium and treated for 6 h before harvesting in 1 × reporter lysis buffer (Promega, Madison, WI, USA). Luciferase activity was measured using the luciferase assay system (Promega).

### *Reverse transcription-PCR*

RNA isolation, reverse transcription, primers, PCR conditions and cycling parameters for glyceraldehyde-3-phos-

phate dehydrogenase (GAPDH) were as described previously (Bergmann *et al.*, 2000). Primer pairs (5' > 3') for the histamine H<sub>1</sub> receptor were AACGGACTCAGATACCAC CA (forward) and TCATTGCACAAGGGGTAGAT (reverse), and cycling parameters were 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 30 cycles. Histamine H<sub>2</sub> receptor primers were TGACCAATTGTTTCA TCGTG (forward) and TGAAG TAGGGAAACCAGCAG (reverse), and cycling parameters were 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 44 cycles. Histamine H<sub>3</sub> receptor primers were CCTCCTCTG CCTCAACATC (forward) and GGAAGGGCGTAAA GAACTCC (reverse), and cycling parameters were 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 44 cycles. Histamine H<sub>4</sub> receptor primers were TCGAATGGGATTTGGAAAG (forward) and TGGCCCATTCACCTAAGAAGG (reverse), and cycling parameters were 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 44 cycles. Following amplification, PCR products (10 µl) were size fractionated on 1.8% agarose gels stained with ethidium bromide.

#### Real-time TaqMan PCR analysis

After cDNA synthesis, TaqMan PCR was performed using 2.5 µl of cDNA in a reaction volume of 20 µl, essentially according to the manufacturer's specification (Applied Biosystems Inc., Foster City, CA, USA) using a pre-made master mix and an ABI 7900HT instrument (Applied Biosystems). Analysis of GAPDH was carried out using the validated off-the-shelf assay 432631E (Applied Biosystems). IL-8 was amplified using the primers (5' > 3') CTGGCCGTGG CTCTCTTG (forward) and TTAGCACTCCTTGCAAAACTG (reverse) with the 5-carboxyfluorescein/5-carboxytetramethylrhodamine-linked probe 5'-CCTTCCTGATTTCTGCA GCTCTGTGTGAA-3'. IL-6 was amplified using the primers (5' > 3') TGGCTGAAAAGATGGATGCT (forward) and AACT CCAAAGACCAGTGATGATT (reverse) with the 5-carboxyfluorescein/minor-groove-binding protein-linked probe CAA TGAGGAGACTTG. All primers were designed using Primer Express version 2 software (Applied Biosystems). Samples were analysed in duplicate and relative cDNA concentrations were determined from a cDNA standard curve that was analysed simultaneously with the test samples.

#### Western blot analysis

Cells were harvested in 1 × reporter lysis buffer (Promega) containing 1 × Complete Protease Inhibitor Cocktail (Roche, Laval, QC, Canada). Samples were run on 4–12% gradient SDS polyacrylamide gels (Invitrogen) and transferred to Hybond-ECL nitrocellulose paper (GE Healthcare Bio-Sciences Inc., Baie d'Urfé, QC, Canada) using standard techniques. Membranes were probed with an anti-GAPDH (no. 4699-9555) (AbD Serotec, Raleigh, NC, USA) and anti- $\text{I}\kappa\text{B}\alpha$  (sc-371) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. Proteins were visualized using ECL (GE Healthcare Bio-Sciences Inc.).

#### Determination of antagonist affinity

Antagonist affinity was determined by nonlinear regression (Lew and Angus, 1995; Motulsky and Christopoulos, 2005).

Each pair of concentration–response curves (that is, the control concentration–response curve and the concentration–response curve constructed in the presence of antagonist) was fitted simultaneously to a form of the Hill and Gaddum–Schild equation (Equation (1)) derived by Waud *et al.* (1978). Thus, where [A] and [B] are the molar concentrations of agonist and antagonist, respectively, *S* is the Schild slope factor and  $pA_2$  is the affinity of the antagonist when *S* = 1, which is equivalent to the  $pK_B$ . To determine whether *S* deviated significantly from unity, the mean family of response (*E*) to concentration ([A]) (that is, *E*/[A]) curves that made up the entire experiment was fitted globally to the Hill and Gaddum–Schild equation (Equation (1)) under two conditions: one where *S* was constrained to a constant equal to 1 and the other where it was a shared value for all data sets (Motulsky and Christopoulos, 2005). The F-test was applied to determine statistically which equation gave the best fit and this was then used for the analysis (Prizm 4, GraphPad Software Inc., San Diego, CA, USA). In each case, *S* did not deviate from unity and, therefore, was constrained to a value of 1.

$$E = E_{\min} + \left[ \frac{(E_{\max} - E_{\min})}{1 + \left[ \frac{10^{\log p[A]_{50}} [1 + \frac{[B]}{10^{-pA_2}}]^S}{[A]} \right]^n} \right] \quad (1)$$

#### Statistics

Data are presented as means ± s.e.m. of *n* independent observations. Comparison between groups of experimental data was performed using either a one-way analysis of variance with a Bonferroni post-test or Student's *t*-test, as appropriate. Significant differences between means are shown as \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001.

#### Materials

TNF $\alpha$  (R&D Systems, Hornby, ON, Canada) and histamine (Sigma, Oakville, ON, Canada) were dissolved in sterile phosphate-buffered saline. Mepyramine (Sigma), tiotidine (Tocris Bioscience, Ellisville, MI, USA), thioperamide (Tocris Bioscience),  $\alpha$ -methylhistamine (Tocris Bioscience) and 4-methylhistamine (Tocris Bioscience) were all dissolved in water; JNJ7777120 (Sigma) was dissolved in dimethylsulphoxide. Final concentrations of dimethylsulphoxide added to cells were <0.1% and this had no effect on any of the responses (data not shown).

## Results

#### Role of histamine and H<sub>1</sub> receptor in pro-inflammatory gene expression

Stimulation of BEAS-2B cells with histamine alone had no significant effect on either IL-6 or IL-8 expression at 1 and 2 h post-stimulation (Table 1). However, at 6 h after histamine stimulation, both IL-6 and IL-8 were significantly induced by 1.9- and 1.8-fold, respectively (Table 1). At 12 and 24 h, while

IL-8 production remained significantly increased over basal levels, histamine-induced IL-6 was not significantly induced (Table 1). In addition, the enhancement of IL-6 and IL-8 release by TNF $\alpha$  in the presence of histamine was maximal at 6 h. In the presence of TNF $\alpha$ , histamine concentration-dependently increased IL-6 ( $EC_{50}=4.69 \times 10^{-7}$  M) and IL-8 ( $EC_{50}=2.59 \times 10^{-7}$  M) release from BEAS-2B cells to a maximum of 7.3- and 2.7-fold, respectively, at 100  $\mu$ M (Figures 1a and c). This enhancement occurred at all concentrations of

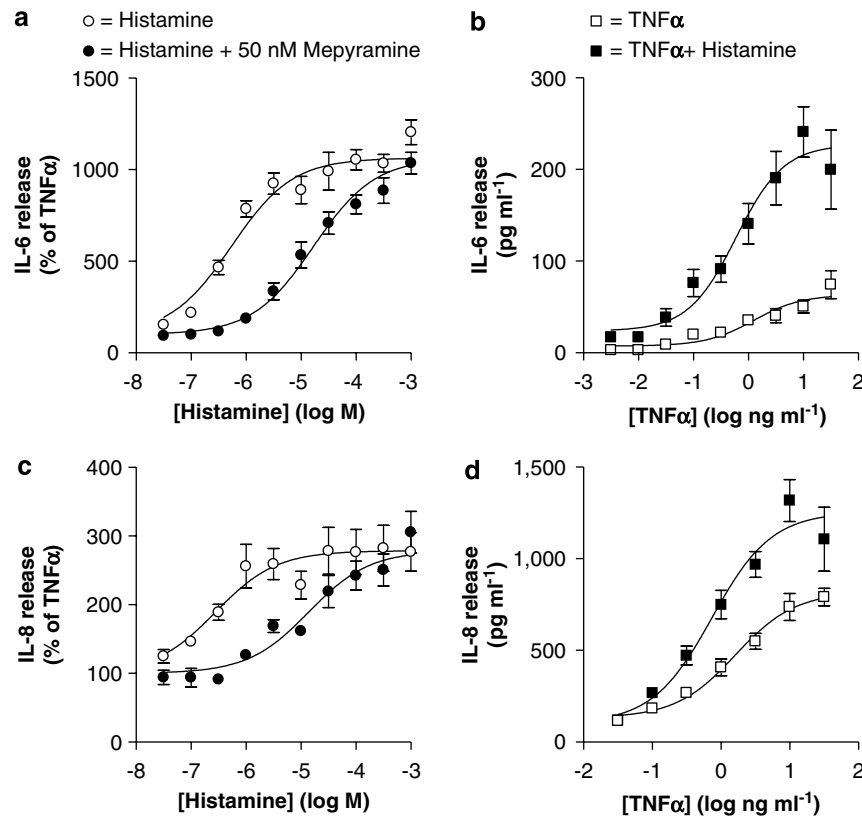
TNF $\alpha$  studied (Figures 1b and d). Prior addition of the H $_1$  receptor antagonist, mepyramine, at 5 nM, had no obvious effect on either IL-6 or IL-8 release (data not shown), whereas at 50 nM, mepyramine resulted in 22- and 45-fold rightward shifts in the  $EC_{50}$  values to histamine for IL-6 and IL-8, respectively, and produced  $pA_2$  values of 8.78 and 8.99, respectively.

To investigate mechanisms involved in histamine-dependent potentiation of IL-6 and IL-8 release, cells were treated

**Table 1** Time-course analysis of IL-6 and IL-8 release in BEAS-2B cells

Time (h)	IL-6 release ( $pg\ ml^{-1}$ )				IL-8 release ( $pg\ ml^{-1}$ )			
	NS	His	TNF $\alpha$	TNF $\alpha$ + His	NS	His	TNF $\alpha$	TNF $\alpha$ + His
1	11.2 ( $\pm 2.7$ )	13.1 ( $\pm 4.9$ )	22.6 ( $\pm 4.4$ )	48.0 ( $\pm 13.0$ )	7.5 ( $\pm 1.4$ )	10.3 ( $\pm 1.2$ )	36.3 ( $\pm 5.0$ )	65.1 ( $\pm 8.3$ )
2	9.4 ( $\pm 2.7$ )	14.6 ( $\pm 2.1$ )	65.9 ( $\pm 11.3$ )	135 ( $\pm 8.1$ )	6.8 ( $\pm 1.3$ )	9.0 ( $\pm 0.5$ )	101 ( $\pm 14.0$ )	172 ( $\pm 14.2$ )
6	10.9 ( $\pm 3.1$ )	21.2** ( $\pm 2.1$ )	180* ( $\pm 35.2$ )	666*** ( $\pm 68.5$ ) <sup>†††</sup>	6.9 ( $\pm 0.7$ )	12.4* ( $\pm 2.5$ )	503*** ( $\pm 57.3$ )	1980*** ( $\pm 351$ ) <sup>†††</sup>
12	22.3 ( $\pm 5.6$ )	33.6 ( $\pm 5.2$ )	796*** ( $\pm 87.0$ )	1130*** ( $\pm 92.4$ ) <sup>†††</sup>	6.5 ( $\pm 1.0$ )	13.7** ( $\pm 1.6$ )	3020*** ( $\pm 328$ )	6043*** ( $\pm 913$ ) <sup>†††</sup>
24	30.7 ( $\pm 5.5$ )	35.7 ( $\pm 3.8$ )	1140*** ( $\pm 129$ )	1310*** ( $\pm 89.0$ )	7.6 ( $\pm 0.2$ )	14.0*** ( $\pm 1.0$ )	2950*** ( $\pm 967$ )	3620*** ( $\pm 257$ ) <sup>†††</sup>

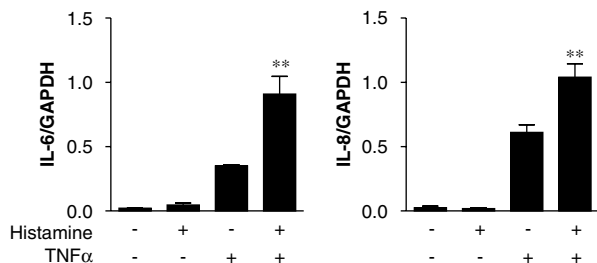
BEAS-2B cells were either not stimulated (NS) or were treated with either histamine (100  $\mu$ M) (His), tumour necrosis factor (TNF)  $\alpha$  (10 ng  $ml^{-1}$ ) or a combination of TNF $\alpha$  and histamine as indicated. Cells were harvested at the indicated times and IL-6 and IL-8 release was measured by enzyme-linked immunosorbent assay. Data ( $n=6$ ) are expressed as means  $\pm$  s.e.m. Significance between NS and each of His, TNF $\alpha$  or TNF $\alpha$  + His is indicated as \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ . Significance between TNF $\alpha$  and TNF $\alpha$  + His is indicated as <sup>†††</sup> $P < 0.001$ .



**Figure 1** Role of histamine and the histamine H $_1$  receptor in tumour necrosis factor (TNF)  $\alpha$ -stimulated release of interleukin (IL)-6 and IL-8. (a and c) BEAS-2B cells were stimulated with TNF $\alpha$  (10 ng  $ml^{-1}$ ) and various concentrations of histamine (0.03–1000  $\mu$ M) in the presence or absence of mepyramine (50 nM). (b and d) BEAS-2B cells were stimulated with various concentrations of TNF $\alpha$  (0.003–30 and 0.03–30 ng  $ml^{-1}$  for IL-6 and IL-8, respectively) in the presence or absence of histamine (100  $\mu$ M). Cells were harvested after 6 h and supernatants were analysed by enzyme-linked immunosorbent assay for IL-6 and IL-8 release. Data ( $n=6$ ) are expressed either as a percentage of cells treated with TNF $\alpha$  alone or as picograms per millilitre. All data are plotted as means  $\pm$  s.e.m. and basal levels of IL-6 and IL-8 were  $5.27 \pm 0.58$  and  $28.52 \pm 2.81$  pg  $ml^{-1}$ , respectively.

with  $\text{TNF}\alpha$  ( $10\text{ ng ml}^{-1}$ ) in the presence or absence of histamine ( $100\ \mu\text{M}$ ). RNA was harvested at 6 h and subjected to real-time PCR. In each case, histamine potentiated the  $\text{TNF}\alpha$ -induced IL-6 and IL-8 mRNA expression 2.6- and 1.7-fold, respectively (Figure 2).

Since the expression of IL-6 and IL-8 is dependent upon the activation of NF- $\kappa\text{B}$  in epithelial cells (Catley *et al.*, 2005; Newton *et al.*, 2007), the effect of histamine on NF- $\kappa\text{B}$ -dependent transcription in BEAS-2B 3 $\kappa\text{Bu}$ -luc reporter cells was examined. Consistent with the cytokine results described above, histamine potentiated NF- $\kappa\text{B}$ -dependent transcription in a concentration-dependent manner ( $\text{EC}_{50} = 1.90 \times 10^{-6}\text{ M}$ ), with a maximum induction of 1.8-fold (Figure 3a). Histamine alone also increased luciferase activity twofold over basal activity and this effect was maintained for all concentrations of  $\text{TNF}\alpha$  (Figure 3b). Pre-incubation (1 h) with 50 nM mepyramine prevented the potentiation of  $\text{TNF}\alpha$ -stimulated luciferase gene expression by histamine at concentrations of less than  $100\ \mu\text{M}$  (Figure 3a). Treatment with 5 nM of mepyramine caused a



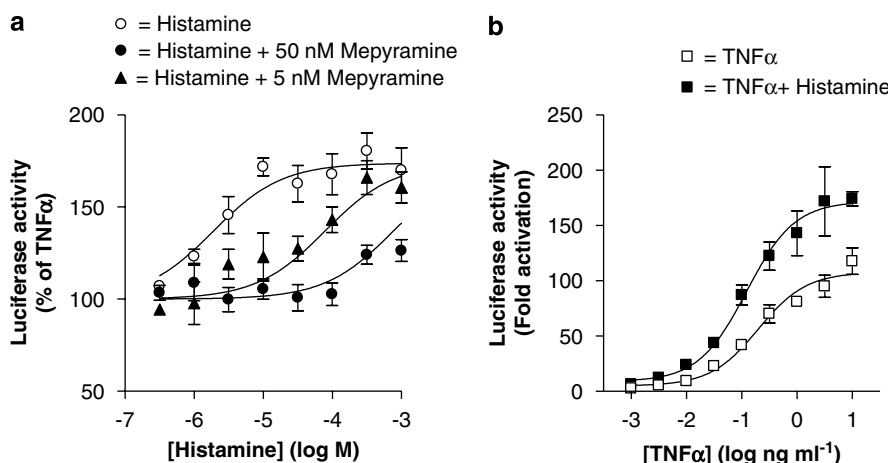
**Figure 2** Effect of histamine on interleukin (IL)-6 and IL-8 mRNA expression. BEAS-2B cells were either left untreated or treated with histamine ( $100\ \mu\text{M}$ ) or tumour necrosis factor ( $\text{TNF}\alpha$ ) ( $10\text{ ng ml}^{-1}$ ), or a combination of histamine ( $100\ \mu\text{M}$ ) plus  $\text{TNF}\alpha$  ( $10\text{ ng ml}^{-1}$ ). Cells were harvested after 6 h and TaqMan real-time PCR was carried out for IL-6, IL-8 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative cDNA concentrations ( $n = 3$ ) were normalized to GAPDH and are plotted as means  $\pm$  s.e.m.

19-fold rightward shift of the histamine response curve (Figure 3a). Analysis of the shift in the  $\text{EC}_{50}$  value for the histamine in the presence of both 50 nM (Figure 3a) and 5 nM (Figure 3a) mepyramine gave rise to a  $\text{pA}_2$  value of 9.91.

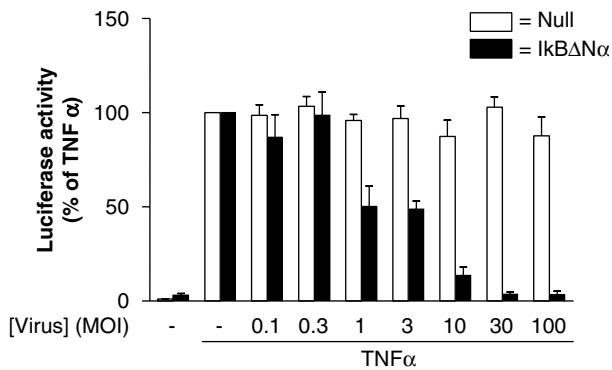
#### Role of NF- $\kappa\text{B}$ in IL-6 and IL-8 release

The role of NF- $\kappa\text{B}$  was investigated using the dominant inhibitor  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  which was delivered using adenoviral-mediated gene transfer. As before, NF- $\kappa\text{B}$ -dependent transcription was strongly induced by  $\text{TNF}\alpha$  ( $10\text{ ng ml}^{-1}$ ) and this was reduced to basal levels by increasing MOIs of the  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$ -expressing virus (Figure 4). A null (empty) adenoviral vector had no significant effect at equivalent MOIs (Figure 4).

To examine the role of NF- $\kappa\text{B}$  in  $\text{TNF}\alpha$ , and  $\text{TNF}\alpha$  plus histamine-induced IL-6 and IL-8 expression, BEAS-2B 3 $\kappa\text{Bu}$ -luc cells were treated with  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  virus or null virus at an MOI of 30. Consistent with the above data,  $\text{TNF}\alpha$ -induced NF- $\kappa\text{B}$  reporter activity was reduced to near basal levels by the  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  adenovirus (Figure 5a). Similarly,  $\text{TNF}\alpha$ -induced release of IL-6 and IL-8 was also significantly reduced by  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$ , which confirmed dependence of these responses on the activation of NF- $\kappa\text{B}$  (Figure 5a). Likewise, cells treated with histamine alone appeared to show small increases in IL-6 and IL-8 release, which decreased in the presence of the  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  adenovirus. However, this was again at the limit of detection and no significance was attained. The addition of Ad5- $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  also significantly repressed  $\text{TNF}\alpha$  plus histamine-induced luciferase activity, IL-6 and IL-8 release (Figure 5a). However, while both luciferase activity and IL-8 were reduced to near basal levels, IL-6 release was reduced by 43% (Figure 5a). In all cases, the null adenovirus was without effect (Figure 5a). Infection of BEAS-2B cells by the  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  adenovirus was confirmed by western blot analysis for  $\text{I}\kappa\text{B}\alpha$  (Figure 5b).  $\text{I}\kappa\text{B}\alpha\Delta\text{N}$  expression was identified as a band of greater electrophoretic mobility than



**Figure 3** Role of histamine and the histamine  $\text{H}_1$  receptor in tumour necrosis factor ( $\text{TNF}\alpha$ )-stimulated nuclear factor (NF)- $\kappa\text{B}$ -dependent transcription. BEAS-2B 3 $\kappa\text{Bu}$  cells were stimulated with (a)  $\text{TNF}\alpha$  ( $10\text{ ng ml}^{-1}$ ) and various concentrations of histamine ( $0.03$ – $1000\ \mu\text{M}$ ) in the presence or absence of either 5 or 50 nM mepyramine and (b) various concentrations of  $\text{TNF}\alpha$  ( $0.001$ – $10\text{ ng ml}^{-1}$ ) in the presence or absence of histamine ( $100\ \mu\text{M}$ ). Cells were harvested after 6 h and lysates analysed for luciferase activity. Data ( $n = 5$ ) are expressed as either a percentage of cells treated with  $\text{TNF}\alpha$  alone or as fold activation of untreated cells. All data are plotted as means  $\pm$  s.e.m.



**Figure 4** Effect of dominant IκBαΔN on nuclear factor (NF)-κB-dependent transcription in BEAS-2B cells. BEAS-2B 3κBu cells were grown to approximately 70% confluence before infection either with a dominant IκBαΔN-expressing adenovirus or a null adenoviral expression vector at the indicated multiplicities of infection (MOI). After 24 h, cells were changed to serum-free media and incubated overnight before being either not stimulated or stimulated with TNFα (10 ng ml<sup>-1</sup>). After 6 h, cells were harvested for luciferase assay. Data (*n* = 6) are plotted as a percentage of tumour necrosis factor (TNF) α-stimulated cells as means ± s.e.m.

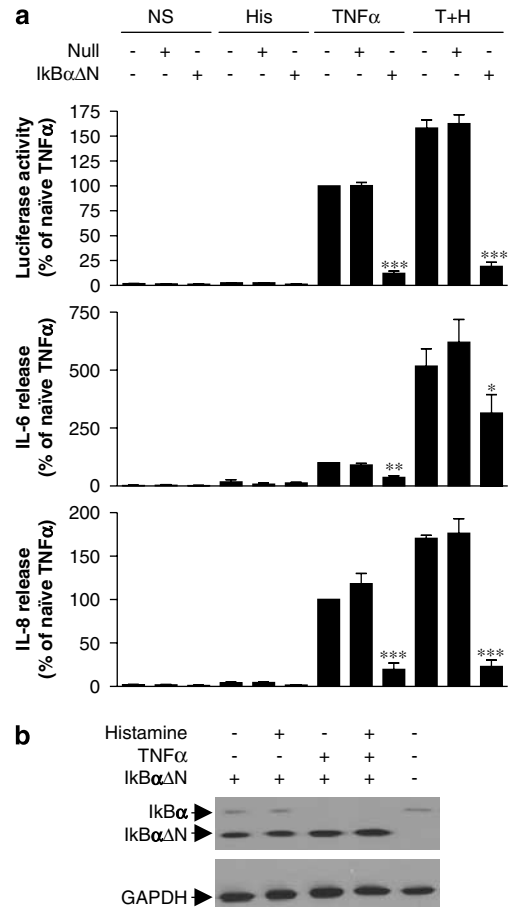
endogenous IκBα (Figure 5b). Additionally, whereas cells treated with TNFα revealed no endogenous IκBα, cells treated with histamine alone exhibited similar IκBα expression to untreated cells, suggesting that histamine had no marked effect on the IκB kinase–IκBα activation pathway (Figure 5b).

#### Expression of histamine receptors in BEAS-2B cells

The expression of histamine receptor mRNA in BEAS-2B cells was determined using conventional end-point reverse transcription-PCR. Analysis of either untreated or TNFα-treated cells revealed constitutive expression of H<sub>1</sub> receptor mRNA (Figure 6). Histamine H<sub>2</sub> receptor mRNA was not detected at 44 cycles of amplification (Figure 6). Despite a DNase digestion step (Qiagen, Mississauga, ON, Canada) in the preparation of RNA, further cycles of amplification resulted in detection of product in both cDNA as well as in RNA samples, which had not been subject to reverse transcription (data not shown). We are, therefore, unable to exclude the possibility that low levels of H<sub>2</sub> receptor mRNA may exist, but are masked by the genomic signal arising from the intronless H<sub>2</sub> receptor gene (see National Centre for Biotechnology Information (NCBI) accession no. NM\_022304). Transcripts encoding the histamine H<sub>3</sub> and H<sub>4</sub> receptor subtypes were also detected in unstimulated cells, but were absent in cells treated with TNFα (10 ng ml<sup>-1</sup>) (Figure 6).

#### No role for the H<sub>2</sub> receptor in the potentiation of NF-κB-dependent transcription and cytokine release

To functionally test for the presence of the G<sub>s</sub>-coupled histamine H<sub>2</sub> receptor subtype, BEAS-2B cells harbouring a CRE-dependent luciferase reporter construct were treated with histamine (from 100 nM to 1 mM). As shown in Figure 7a, histamine activated CRE-dependent transcription in a concentration-dependent manner



**Figure 5** Role of nuclear factor (NF)-κB in the histamine potentiation of luciferase activity and interleukin (IL)-6 and IL-8 release. BEAS-2B 3κBu cells grown to 70% confluence in 24-well plates were either left uninfected or were infected at a multiplicity of infection (MOI) of 30 with either a dominant IκBαΔN-expressing adenovirus or a null adenoviral expression vector. After 24 h, cells were changed to serum-free media and incubated overnight before being stimulated with either histamine (100 μM) (His), TNFα (10 ng ml<sup>-1</sup>) or a combination of TNFα (10 ng ml<sup>-1</sup>) plus histamine (100 μM) (T+H) as indicated. After 6 h, cells were harvested and supernatants were analysed for IL-6 and IL-8 expression. Cell lysates were analysed for (a) luciferase activity and (b) expression of IκBα and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by western blot. Data (*n* = 6) are plotted as a percentage of TNFα-stimulated naïve cells as means ± s.e.m. and basal levels of IL-6 and IL-8 were 7.61 ± 0.91 and 13.62 ± 2.41 pg ml<sup>-1</sup>, respectively.

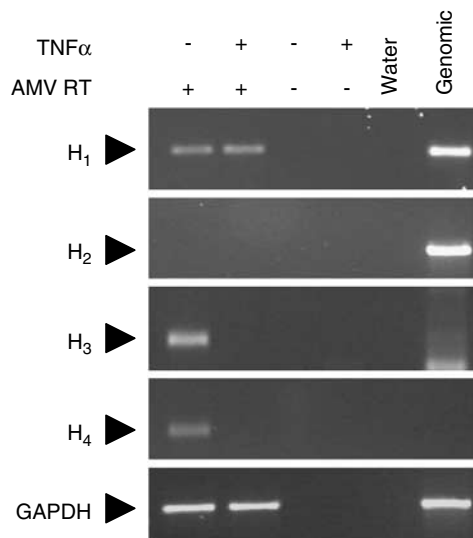
(EC<sub>50</sub> = 1.41 × 10<sup>-5</sup> M), with a maximum response of 2.4-fold (Figure 7a). In contrast, the β<sub>2</sub>-adrenoceptor agonist salbutamol stimulated luciferase activity by 13- or 14-fold, indicating that the responsiveness of BEAS-2B cells to histamine was in fact relatively modest (Figure 7b). Prior addition of the histamine H<sub>2</sub> receptor-selective antagonist, tiotidine (5 μM) to either unstimulated cells or cells treated with salbutamol had no significant effect on CRE-dependent transcription (Figure 7b). Conversely, histamine-stimulated CRE-dependent transcription was significantly inhibited to a level that was not significantly different from basal level by the prior addition of tiotidine to cells (Figure 7b).

To investigate a role for H<sub>2</sub>-dependent signalling in the potentiation of NF-κB-dependent transcription by histamine

and on the release of IL-6 and IL-8, BEAS-2B cells were treated with TNF $\alpha$  and increasing concentrations of histamine in the presence or absence of tiotidine (5  $\mu$ M). Tiotidine was without any significant effect on any of these histamine-induced responses (Figure 8).

*Role of histamine H<sub>3</sub> and H<sub>4</sub> receptors in potentiating NF- $\kappa$ B-dependent transcription and cytokine release*

The effect of H<sub>3</sub> and H<sub>4</sub> receptors in mediating the effects of histamine was investigated using the dual-selective H<sub>3</sub>/H<sub>4</sub>



**Figure 6** Expression of histamine receptors in BEAS-2B cells. BEAS-2B cells were either not stimulated or were treated with tumour necrosis factor (TNF)  $\alpha$  (10 ng ml<sup>-1</sup>). After 6 h, RNA was isolated and reverse transcription-PCR in the presence or absence of the AMV reverse transcriptase (AMV RT), as indicated, was carried out using primers for the histamine H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub> receptors as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In addition, water and genomic DNA were analysed in parallel. Ethidium bromide-stained gels representative of three such experiments are shown.

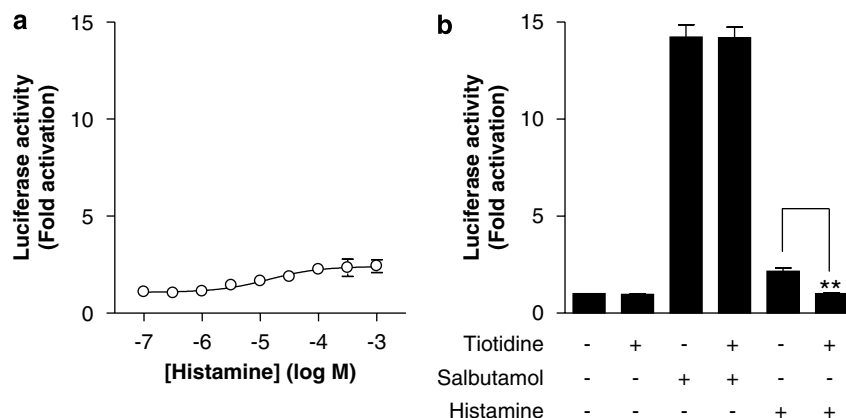
receptor antagonist thioperamide and the selective H<sub>4</sub> receptor antagonist JNJ7777120. BEAS-2B 3 $\kappa$ Bu-luc cells were stimulated with 10 ng ml<sup>-1</sup> of TNF $\alpha$  in the presence of 30  $\mu$ M histamine. Co-incubation with increasing concentrations of thioperamide or JNJ7777120 had no significant effect on luciferase activity or on IL-6 and IL-8 release (Figure 9a). However, stimulation with TNF $\alpha$  and high concentrations (30–100  $\mu$ M) of either the selective H<sub>3</sub> receptor agonist R $\alpha$ -methylhistamine or the selective H<sub>4</sub> receptor agonist 4-methylhistamine significantly increased TNF $\alpha$ -induced release of IL-6 and IL-8 (Figure 9b). Neither R $\alpha$ -methylhistamine nor 4-methylhistamine agonist promoted NF- $\kappa$ B-dependent luciferase activity (Figure 9b).

*Effect of the glucocorticoid dexamethasone on NF- $\kappa$ B, IL-6 and IL-8*

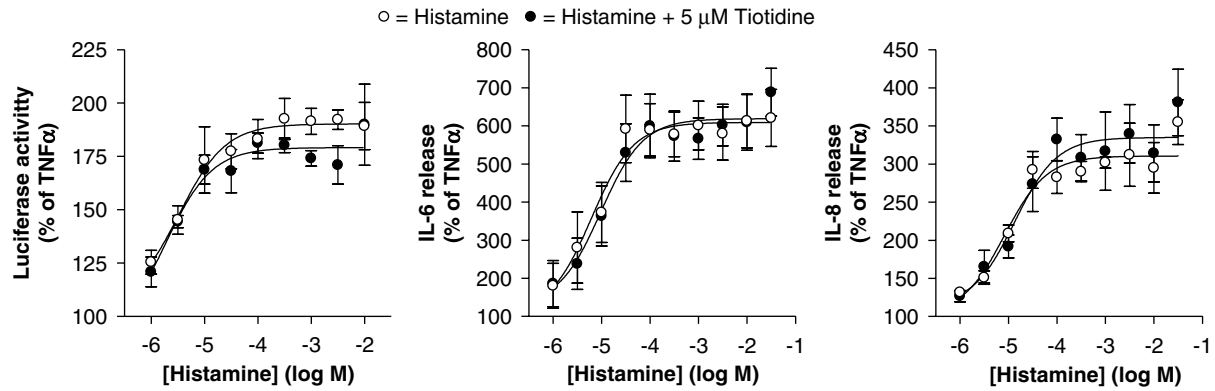
To examine the effect of anti-inflammatory glucocorticoids on histamine-enhanced inflammatory responses, BEAS-2B cells were pretreated with a maximally effective concentration (1  $\mu$ M) of dexamethasone prior to stimulation with TNF $\alpha$  and histamine. Under these conditions, NF- $\kappa$ B-dependent transcription induced by TNF $\alpha$  or TNF $\alpha$  plus histamine was decreased by 33 and 44%, respectively (Figure 10). In contrast, inhibition of both IL-6 and IL-8 release was considerably greater, with TNF $\alpha$  and TNF $\alpha$  plus histamine-induced IL-6 being repressed by 65 and 70%, respectively, whereas IL-8 release was reduced by ~90% for both treatments (Figure 10).

**Discussion and conclusions**

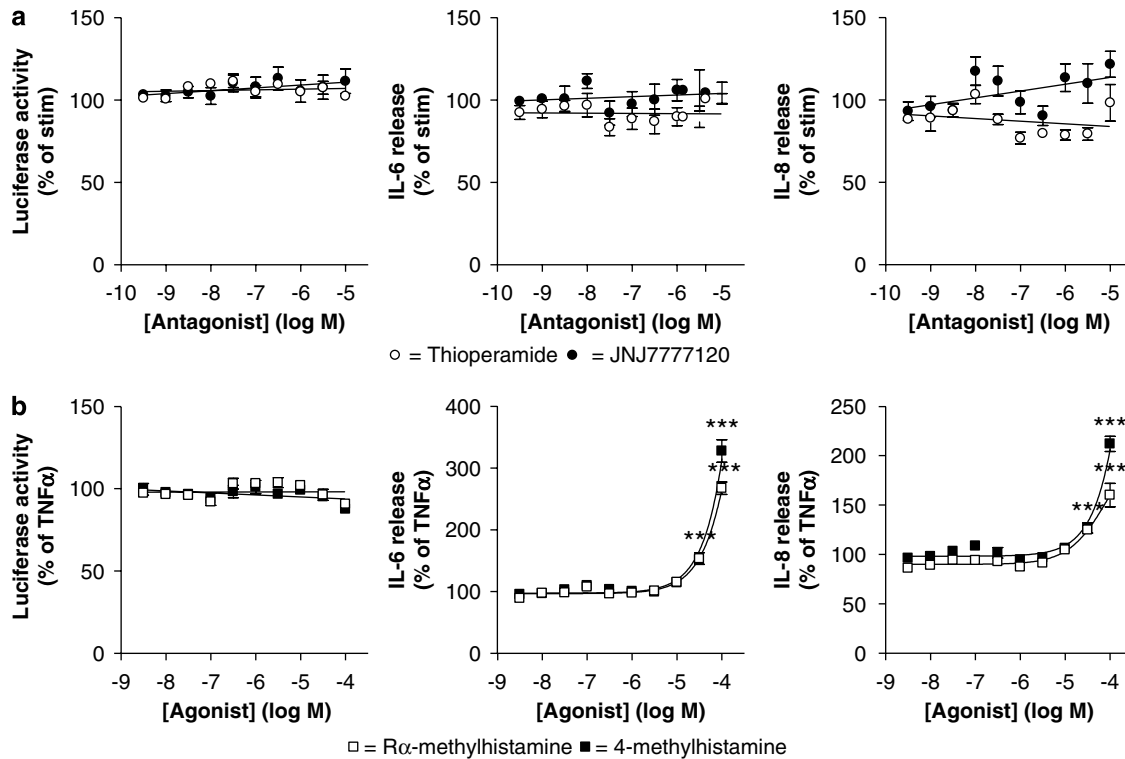
Previous studies in several different cell types have demonstrated NF- $\kappa$ B activation and release of cytokines elicited by histamine acting via the H<sub>1</sub> receptor subtype (Bruysters *et al.*, 2004; Wu *et al.*, 2004; Matsubara *et al.*, 2005; Jin *et al.*, 2006; Muller *et al.*, 2006). However, whereas inflammation is complex and involves multiple mediators, including



**Figure 7** Effect of histamine or a  $\beta_2$ -adrenoceptor agonist on cyclic AMP response element (CRE)-dependent transcription. (a) BEAS-2B CRE reporter cells were either left unstimulated or were stimulated with various concentrations of histamine (0.1–1000  $\mu$ M). (b) BEAS-2B CRE reporter cells were either left unstimulated or were stimulated with either salbutamol (1  $\mu$ M) or histamine (100  $\mu$ M) in the presence or absence of tiotidine (5  $\mu$ M). In each case, cells were harvested after 6 h and luciferase activity was analysed. Data ( $n = 3$ –5) are plotted as the fold activation of unstimulated cells as means  $\pm$  s.e.m.



**Figure 8** Effect of the histamine  $H_2$  receptor antagonist tiotidine on the histamine potentiation of nuclear factor (NF)- $\kappa$ B-dependent transcription and release of interleukin (IL)-6 and IL-8. BEAS-2B  $3\kappa$ Bu cells were incubated in the presence or absence of  $5\mu\text{M}$  tiotidine. After 1 h, cells were stimulated with tumour necrosis factor (TNF)  $\alpha$  ( $10\text{ ng ml}^{-1}$ ) in the presence of increasing concentrations of histamine. Cells were harvested after 6 h and lysates were analysed for luciferase activity. In addition, supernatants were analysed for IL-6 and IL-8 release. Data ( $n=4-7$ ) are plotted as a percentage of TNF $\alpha$ -stimulated cells as means  $\pm$  s.e.m. Basal levels of IL-6 and IL-8 were  $6.33 \pm 0.54$  and  $34.41 \pm 1.91\text{ pg ml}^{-1}$ , respectively.

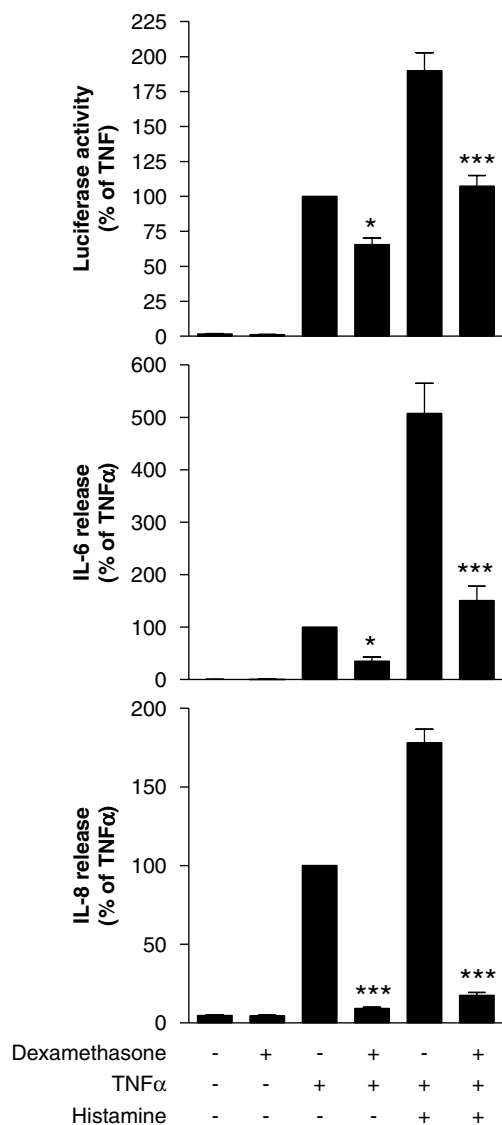


**Figure 9** Role of the  $H_3$  and  $H_4$  histamine receptors in the histamine potentiation of nuclear factor (NF)- $\kappa$ B-dependent transcription and interleukin (IL)-6 and IL-8 release. (a) BEAS-2B  $3\kappa$ Bu cells were incubated in the presence or absence of various concentrations of either thioperamide or JNJ777120. After 1 h, cells were stimulated with a combination of tumour necrosis factor (TNF)  $\alpha$  ( $10\text{ ng ml}^{-1}$ ) plus histamine ( $30\mu\text{M}$ ). (b) Cells were treated with TNF $\alpha$  ( $10\text{ ng ml}^{-1}$ ) and increasing concentrations of either  $R\alpha$ -methylhistamine or 4-methylhistamine. Cells were harvested after 6 h and lysates were analysed for luciferase activity. In addition, supernatants were analysed for IL-6 and IL-8 release. Data ( $n=4-7$ ) are expressed (a) as a percentage of TNF $\alpha$  plus histamine-stimulated cells, where basal levels of IL-6 and IL-8 were  $7.52 \pm 1.42$  and  $16.03 \pm 3.04\text{ pg ml}^{-1}$ , respectively, or (b) as a percentage of TNF $\alpha$ -stimulated cells, where basal levels of IL-6 and IL-8 were  $8.09 \pm 0.74$  and  $13.04 \pm 0.93\text{ pg ml}^{-1}$ , respectively. All data are plotted as means  $\pm$  s.e.m.

histamine and pro-inflammatory cytokines, most investigations have examined the effects of histamine acting alone. In the current study, we examined the ability of histamine to enhance pro-inflammatory responses in BEAS-2B cells elicited by TNF $\alpha$  as a representative, but key, inflammatory

cytokine. Thus, as described by Muller *et al.* (2006), stimulation of BEAS-2B cells with histamine alone induced IL-6 and IL-8 production. However, this response was very modest when compared with cells treated with TNF $\alpha$ . Likewise, histamine alone produced little or no activation





**Figure 10** Effect of dexamethasone on the histamine potentiation of nuclear factor (NF)- $\kappa$ B-dependent transcription and on the release of interleukin (IL)-6 and IL-8. BEAS-2B 3 $\kappa$ Bu cells were incubated in the presence or absence of dexamethasone (1  $\mu$ M) for 1 h before stimulation with either tumour necrosis factor (TNF)  $\alpha$  (10 ng ml $^{-1}$ ) or TNF $\alpha$  (10 ng ml $^{-1}$ ) plus histamine (100  $\mu$ M). Cells were harvested after 6 h and lysates were analysed for luciferase activity. In addition, supernatants were analysed for IL-6 and IL-8 release. Data ( $n = 5-12$ ) are expressed as a percentage of cells treated with TNF $\alpha$  alone as means  $\pm$  s.e.m. Basal levels of IL-6 and IL-8 were 7.80  $\pm$  0.69 and 21.57  $\pm$  1.26 pg ml $^{-1}$ , respectively.

of an NF- $\kappa$ B reporter construct, yet significantly (synergistically) enhanced both TNF $\alpha$ -induced NF- $\kappa$ B-dependent transcription and TNF $\alpha$ -induced release of IL-6 and IL-8 at 6 h. For IL-6 and IL-8 release, this effect was maximal at 6 h, whereas by 24 h, the histamine-dependent enhancement had disappeared for IL-6 and was greatly reduced for IL-8. Reasons for this are not presently clear, but it is possible that histamine, in addition to stimulating positive regulatory processes, may also lead to enhanced feedback control processes and these may attenuate the responses with increasing time.

Similar enhancement phenomena have been described in coronary artery endothelial cells where TNF $\alpha$ - and LPS-induced IL-6 and IL-8 release was also enhanced by histamine (Li *et al.*, 2001). As in our present study, maximal responses were achieved at  $\sim$ 10  $\mu$ M histamine and these were blocked by the H $_1$  receptor antagonist, diphenhydramine but not the H $_2$  receptor antagonist famotidine. Likewise, lipoteichoic acid- and peptidoglycan-induced IL-6 release was also increased by histamine in a diphenhydramine-sensitive, but famotidine-insensitive manner (Talreja *et al.*, 2004). In both these studies, the enhancing effect of histamine was mediated through the H $_1$  receptor subtype and involved NF- $\kappa$ B-dependent transcription. However, in our current study, the apparent affinity of the H $_1$  receptor antagonist mepyramine was  $\sim$ 10-fold higher for blocking histamine-induced NF- $\kappa$ B-dependent transcription ( $pA_2 = 9.91$ ) than for suppressing the release of IL-6 and IL-8 ( $pA_2 = 8.78$  and 8.99, respectively). The  $pA_2$  of mepyramine on NF- $\kappa$ B-dependent transcription is broadly consistent with previously published values (Tayo and Bevan, 1986; Impicciatore *et al.*, 1987; Kamei *et al.*, 1990; Djuric and Andjelkovic, 1995). However, the lower affinity of mepyramine, when IL-6 and IL-8 release is used as a measure, suggests that while the enhancement of NF- $\kappa$ B-dependent transcription is entirely H $_1$  receptor-dependent, the enhancement by histamine of IL-8 and, particularly, IL-6 release may also involve additional histamine receptor subtypes. This supposition is supported by examining the role of NF- $\kappa$ B in the histamine-dependent enhancement of IL-6. Thus, the dominant inhibitor I $\kappa$ B $\alpha$ DN completely suppressed NF- $\kappa$ B-dependent transcription and IL-8 release, but only inhibited IL-6 release by  $\sim$ 50% in cells stimulated with TNF $\alpha$  plus histamine. Analysis of the IL-6 promoter has revealed not only the presence of an NF- $\kappa$ B site (Liebermann and Baltimore, 1990) but also interferon regulatory factor-1 (IRF-1)- (Faggioli *et al.*, 1997), activator protein-1 (AP-1)- (Dendorfer *et al.*, 1994), CCAAT/enhancer-binding protein (C/EBP)- (Isshiki *et al.*, 1990; Akira and Kishimoto, 1997) and Sp1-binding sites (Kang *et al.*, 1996; Armenante *et al.*, 1999), through which histamine could potentially act. In addition, histamine potentiation of IL-6 mRNA was no more than 3-fold compared to 5-7-fold for protein release. Thus, histamine may also modulate IL-6 release via post-transcriptional processes, including changes in mRNA stability, translation or secretion.

In the current study, mRNA for the histamine H $_1$  receptor was constitutively expressed and was the most readily detectable, suggesting that the H $_1$  receptor is the most abundant subtype expressed by BEAS-2B cells. In contrast, our inability to detect mRNA for the H $_2$  receptor gene correlated with a lack of effect of tiotidine on the histamine-dependent enhancements of NF- $\kappa$ B, IL-6 and IL-8. However, this does not preclude the existence of H $_2$  receptors on the cell, by virtue of low levels of H $_2$  receptor mRNA. Indeed, histamine activation of the CRE reporter construct, and inhibition by tiotidine, suggests that low levels of H $_2$  receptor may be present. Analysis of H $_3$  and H $_4$  receptor mRNA revealed basal expression that was profoundly down-regulated by TNF $\alpha$ . These data suggest the existence of novel negative control mechanisms. In this respect, the

downregulation of H<sub>4</sub> receptor mRNA was particularly surprising, since promoter analysis reveals binding sites for inflammatory factors, including both AP-1 and NF- $\kappa$ B (Coge *et al.*, 2001).

Despite the lack of effect of H<sub>3</sub> and H<sub>4</sub> receptor antagonists on the histamine enhancement of inflammatory gene expression, this does not necessarily exclude a role for these receptors. Indeed, we observed that both H<sub>3</sub> and H<sub>4</sub> agonists, at high concentrations, increased the release of IL-6 and IL-8 in TNF $\alpha$ -stimulated cells. Likewise, a number of reports suggest a pro-inflammatory role of the H<sub>4</sub> receptor (O'Reilly *et al.*, 2002; Ling *et al.*, 2004; Zhang *et al.*, 2006), including the release of IL-6 in the mouse (Dunford *et al.*, 2006). In contrast, activation of the histamine H<sub>3</sub> receptor is mainly linked to anti-inflammatory effects, including inhibition of substance P release (Ohkubo *et al.*, 1995), of neurogenic vascular inflammation (McLeod *et al.*, 1998) and of paw oedema (Rouleau *et al.*, 2000). In the current study, the concentrations of histamine H<sub>3</sub> and H<sub>4</sub> receptor agonists that elicited upregulation of IL-6 and IL-8 release were at the point where their selectivity of action must be called into question. Indeed, 4-methylhistamine, the selective H<sub>4</sub> receptor agonist, which had the greatest effect on IL-6 and IL-8 release, has been shown in agonist displacement assays to have effects on not only the H<sub>4</sub> receptor but also via H<sub>2</sub> and H<sub>3</sub> receptors, and at concentrations of 30–100  $\mu$ M via the H<sub>1</sub> receptor (Lim *et al.*, 2005).

Given the above data, how does one reconcile the suggested involvement of other receptors in addition to H<sub>1</sub> receptor-mediated events in the enhancement of IL-6 and IL-8 release? A number of possibilities for this discrepancy do exist and have been reported previously. First, the histamine H<sub>3</sub> receptor exists as at least six splice variants (Wellendorph *et al.*, 2002). In addition, the relatively newly discovered histamine H<sub>4</sub> receptor was found through its homology to the H<sub>3</sub> receptor and may also be expected to exist as multiple isoforms (although to date there are little data addressing this possibility). Furthermore, much of the data relating to H<sub>3</sub> and H<sub>4</sub> receptor agonists and antagonists relies upon transfected reporter systems and the overexpression of specific receptor isoforms (for example, see Lim *et al.*, 2005). Thus, uncharacterized effects of agonists and antagonists against different receptor isoform variants could account for our current observations (Wellendorph *et al.*, 2002). Second, it has been suggested that rather than existing as monomers, G-protein-coupled receptors may exist as homo- or heterodimers or even higher order oligomers (see Milligan, 2004 for review). Thus it is possible to speculate that as with other G-protein-coupled receptors, histamine receptors may also exist as dimers and differential dimerization could lead to nonstandard effects of agonists and antagonists.

In previous studies, we hypothesized the existence of a protein kinase C-dependent pathway, which potentiated TNF $\alpha$ -stimulated NF- $\kappa$ B-dependent transcription in A549 and BEAS-2B cells via enhanced transactivation rather than via increased NF- $\kappa$ B DNA binding (Catley *et al.*, 2004). Since activation of the H<sub>1</sub> receptor results in the production of diacylglycerol and release of calcium, both of which activate protein kinase C, the H<sub>1</sub>-dependent enhancement of NF- $\kappa$ B-

dependent transcription described in the current study may also act through this same pathway. In addition, the finding that histamine-enhanced IL-6 expression is not completely NF- $\kappa$ B-dependent suggests additional mechanisms of potentiation. In terms of inflammatory responses, all these effects may be expected to enhance inflammation as a result of the increases in both IL-6 and IL-8 expression. While these data indicate the possible therapeutic utility of blockade of the histamine H<sub>1</sub> receptor, we also find that the anti-inflammatory glucocorticoid dexamethasone is effective in reducing these enhanced responses. Therefore, our data suggest that the enhanced production of inflammatory mediators, in this case IL-6 and IL-8, will be effectively targeted by existing anti-inflammatory strategies that involve glucocorticoids.

In summary, we have documented a histamine-dependent increase in both TNF $\alpha$ -stimulated NF- $\kappa$ B-dependent transcription and the release of the pro-inflammatory mediators IL-6 and IL-8. These effects were determined to be wholly dependent, in the case of NF- $\kappa$ B-dependent transcription, and predominantly dependent, in the case of IL-6 and IL-8, on the histamine H<sub>1</sub> receptor. In addition, the histamine-dependent enhancement of NF- $\kappa$ B-dependent transcription and IL-8 release was completely ablated by the dominant I $\kappa$ B $\alpha$  $\Delta$ N, whereas IL-6 release was only partially inhibited, suggesting the existence of additional regulatory steps aside from NF- $\kappa$ B. However, antagonists of the H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub> histamine receptors had no impact on the histamine enhancement, and selective stimulation of the H<sub>3</sub> and H<sub>4</sub> receptors was effective at enhancing IL-6 and IL-8 release only at concentrations that may yield nonspecific effects. Finally, despite showing that histamine can contribute to the inflammatory response by increasing NF- $\kappa$ B activation and inflammatory cytokine release, these effects are effectively combated by glucocorticoids, suggesting that existing anti-inflammatory therapies may already repress these enhanced responses due to histamine.

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## Conflict of interest

RN and MAG have received research funding from Glaxo-SmithKline, AstraZeneca and Altana Pharmaceuticals. The authors are unaware of any other conflicts of interest.

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