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Glucocorticoid-mediated transrepression is regulated by histone acetylation and DNA methylation

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Abstract

Glucocorticoids are highly effective in controlling chronic inflammatory diseases by inhibiting the expression of cytokines and chemokines. Glucocorticoids act through binding of their receptor resulting to inhibition of transcription factors such as nuclear factor kappaB (NF-κB). This may occur via the transcription integrator protein, CREB binding protein (CBP), which has intrinsic histone acetylase (HAT) activity. Interleukin (IL)-1β caused a significant increase in NF-κB-mediated granulocyte/macrophage colony stimulating factor (GM-CSF) release, which was inhibited by the glucocorticoid mometasone furoate (MF) ($EC_{50} = 2 \times 10^{-11}$ M). This effect was inhibited by CBP over-expression. The role of histone acetylation and DNA methylation in the transcription of GM-CSF was indicated by trichostatin A (TSA), an inhibitor of histone deacetylases, and 5-azacytidine (5-aza), a DNA methylase inhibitor, to increase GM-CSF expression partially blocking glucocorticoid inhibition of IL-1β-stimulated GM-CSF release. These data suggest that the mechanism of glucocorticoid action in suppressing interleukin-1β-stimulated GM-CSF release in A549 cells may involve modulation of CBP-mediated histone-acetylase activity and DNA methylation.

Keywords: CREB binding protein; Glucocorticoid receptor; NF-κB (Nuclear factor kappaB); Histone acetylation; DNA methylation; Inflammation

1. Introduction

Inflammation is a central feature of many lung diseases including asthma (Barnes et al., 1988). The inflammatory response involves a complex cascade of mediators whose expression is enhanced during the disease process. Since many of these inflammatory genes are not expressed in normal cells under resting conditions, the increased expression of these proteins must result from enhanced gene transcription occurring in a cell-specific manner (Barnes et al., 1988).

Cytokines, such as interleukin (IL)-1β, tumour necrosis factor-α (TNF-α) and granulocyte/macrophage colony stimulating factor (GM-CSF), play an important role in chronic inflammation (Barnes and Adcock, 1993) and produce their cellular effects by activation of various transcription factors such as activator protein-1 (AP-I) and nuclear factor kappaB (NF-κB) (Latchman, 1996; Barnes, 1994). More recently, AP-1 and NF-κB have been shown to act through the mediation of larger co-activator molecules, such as CREB binding protein (CBP) and p300, which form a bridge between the activated transcription factor and the basal transcription machinery including RNA polymerase II (Chakravarti et al., 1996; Kamei et al., 1996; Janknecht and Hunter, 1996). Furthermore, this interaction may regulate the intrinsic histone acetylase (HAT) activity of CBP (Ogryzko et al., 1996; Wolffe, 1997).

Glucocorticoid receptor (GR) interaction with AP-1 and NF-κB is believed to result in mutual inhibition and account for most of the anti-inflammatory effects of glucocorticoids (Karin, 1998). As such, GR and the retinoic acid receptor-α are able to inhibit AP-1-mediated transcription through an effect on histone acetylation (Kamei et al., 1996; Heery et al., 1997). Histone acetylation is an active process whereby small changes in histone acetyltransferase activity or deacetylase activity can markedly affect the overall histone acetylase activity associated with inflammatory genes (Imhof and Wolffe, 1998). Thus, hyperacetylation and increased gene expression may result from increased histone acetylase activation or a reduction in deacetylase activity or expression.

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Methylation of cytosine residues is also involved in transcriptional silencing by modulating the action of transcriptional activators and by recruitment of repressor proteins (Bird and Wolffe, 1999). This results in the formation of an inactive chromatin complex and subsequent decreased gene expression. Inhibition of methylase activity would therefore be expected to enhance transcription of susceptible genes (Bird and Wolffe, 1999). The actions of DNA methylation are revealed following stimulation of cells with activators with subsequent changes in chromatin structure (Bird and Wolffe, 1999). This may allow the recruitment of additional co-activators or transcription factors to the gene-specific transcriptional complex.

The objective of this study was to investigate the effect of inhibition of histone deacetylases, using trichostatin A (TSA) (Yoshida et al., 1995), and DNA methylation, using 5-azacytidine (5-aza) (Friedman, 1979), on the ability of interleukin-1β to induce GM-CSF release and its subsequent inhibition by dexamethasone (Dex). In addition, we have examined the ability of a histone acetyltransferase, CBP, to modify the actions of mometasone furoate (MF), a synthetic glucocorticoid, on interleukin-1β-stimulated GM-CSF release.

2. Materials and methods

2.1. Cell culture

A549 cells were grown as previously described (Newton et al., 1996). Cells were stimulated by 1 ng/ml interleukin-1β in the presence or absence of dexamethasone or mometasone furoate. The effect of the NF-κB inhibitors phenylarsine oxide (PAO), pyrrolidine dithiocarbamate (PDTC) and the NF-κB cell permeable inhibitor peptide SN50 on GM-CSF release was examined along with the effect of the proteasome inhibitor MG-132 (Schreck et al., 1992). The effects of the histone deacetylase inhibitor trichostatin A (Yoshida et al., 1990) and the DNA demethylation agent 5-azacytidine (Lee et al., 1995) on interleukin-1β-stimulated expression of GM-CSF release was measured in the presence and absence of dexamethasone or mometasone furoate.

2.2. GM-CSF release

GM-CSF concentrations in the culture supernatant were measured using a specific enzyme-linked immunosorbant assay (ELISA) calibrated with human recombinant GM-CSF (0–2000 pg/ml, PharMingen, Lugano, Switzerland).

2.3. CBP over-expression

Cells were grown to confluence and then treated for 2 days in serum-free media. pRc/RSVmCBP8.0 plasmid DNA (Kwok et al., 1994) containing the cDNA sequence for murine CBP was incubated with 2.5 μl TFx50 reagent (Promega)/μg DNA/ml of serum-free media for 15 min at room temperature. Cells were transfected by the addition of 1 ml of media containing DNA–Tfx50 (5 μl Tfx50/μg DNA) for 1.5 h before washing in fresh media and incubation in 1 ml serum-free media. After 18–24 h, the media was changed and cells were stimulated with 1 ng/ml of interleukin-1β in the presence or absence of varying doses of mometasone furoate. The culture medium was removed after 24 h and GM-CSF levels were measured by ELISA.

2.4. Immunoprecipitation and Western blotting

A549 cells were treated with a combination of 10^{-9} M mometasone furoate and 1 ng/ml interleukin-1β for 30 min before total cellular proteins were extracted from A549 cells by freeze–thawing samples in lysis buffer (20 mM HEPES, 1.5 mM $MgCl_2$, 0.42 mM NaCl, 0.5 mM dithiothreitol, 25% glycerol, 0.5 mM phenylmethylsulphonyl fluoride, 0.2 mM EDTA) (Adcock et al., 1996). Immunoprecipitation and Western blotting were performed as described previously (Ito et al., 2000).

2.5. CBP attenuates mometasone furoate inhibition of 6× κB-luciferase activity

Cells were grown to confluence and then treated for 2 days in serum-free media and pEnh-TK-Luc plasmid DNA (Bachelerie et al., 1991) containing 6× human immunodeficiency virus (HIV). κB sites were incubated with 2.5 μl TFx50 reagent (Promega)/μg DNA/ml of serum-free media for 15 min at room temperature. Cells were transfected by the addition of 1 ml of media containing DNA–Tfx50 (5 μl Tfx50/μg DNA) for 1.5 h before washing in fresh media and incubation in 1 ml serum-free media. All cells were co-transfected with 1 μg pSV-β-gal vector (Promega) to control for transfection efficiency. After 18 h, the media was changed and cells were stimulated with varying doses of mometasone furoate in the presence or absence of 1 ng/ml of interleukin-1β. Cells were harvested, by scraping, and resuspended in 1× reporter Lysis Buffer (Promega). After incubation at room temperature for 15 min, lysates were vortexed for 10 s and subjected to one freeze–thaw cycle. Cellular debris was pelleted and total protein was measured. Luciferase assays were performed using 20 μl of extract and 50 μl Luciferase Assay Reagent (Promega) and luminescence was measured with a TD 20/20 Luminometer (Turner Designs, Hemel Hempstead, UK). Relative luminescence readings were normalised to β-galactosidase expression and expressed as a percentage of activation relative to control or interleukin-1β-stimulated release. CBP over-expression was confirmed by Western blot analysis of cell lysates.

2.6. Histone extraction and acetylation activity

Cells which were plated at a density of 0.25×10^6 cells/ml were exposed to 0.05 mCi/ml of [^{3}H] acetate (Amersham). After incubation for 10 min at 37 °C, cells were stimulated with various drugs for 6 h. Histones were then isolated and acetylation activity were analysed as previously described (Ito et al., 2000). Protein concentrations of the histone-containing supernatant were determined by Bradford protein assay kit (BioRad, Hemel Hempstead, UK). Radioactivity in extracted histones was determined by liquid scintillation counting and expressed per nanogram protein.

2.7. Data analysis

Data are expressed as mean ± standard error of the mean (S.E.M.) of *n* independent observations. EC_{50} values (concentration of drug required to elicit 50% of the maximal inhibition) were calculated by using non-linear iterative regression with the 'PRISM' curve fitting programme (GraphPad Instat™ software programme). A multiple comparison was made between the mean of the control and the means from each individual treatment group by Dunnett's test using SAS/STAT software (SAS Institute, Cary, NC, USA). All statistical testings were performed using a two-sided 5% level of significance.

3. Results

3.1. The role of NF-κB in GM-CSF release

Interleukin-1β produced a dose-dependent increase in GM-CSF release which reached a plateau at 1 ng/ml (pre: 43 ± 11 pg/ml and post: 1136 ± 59 pg/ml) after 24 h (Fig. 1A). Interleukin-1β (1 ng/ml)-stimulated release of GM-CSF was inhibited in a concentration-dependent manner by the NF-κB inhibitors phenylarsine oxide, which was maximal at 10 μM, and 1 mM pyrrolidine dithiocarbamate (Fig. 1B). Further, evidence for a role of NF-κB in GM-CSF release was shown by inhibition of proteasome activity by MG-132. MG-132 produced up to a 70% suppression of GM-CSF release with an EC_{50} of 25 μM (Fig. 1B). SN50, an NF-κB nuclear localisation site-blocking peptide, blocked interleukin-1β-induced GM-CSF release, while the control peptide SN50N had no effect (Fig. 1C). Dexamethasone ($EC_{50} = 2.2 \times 10^{-9}$ M) and mometa-

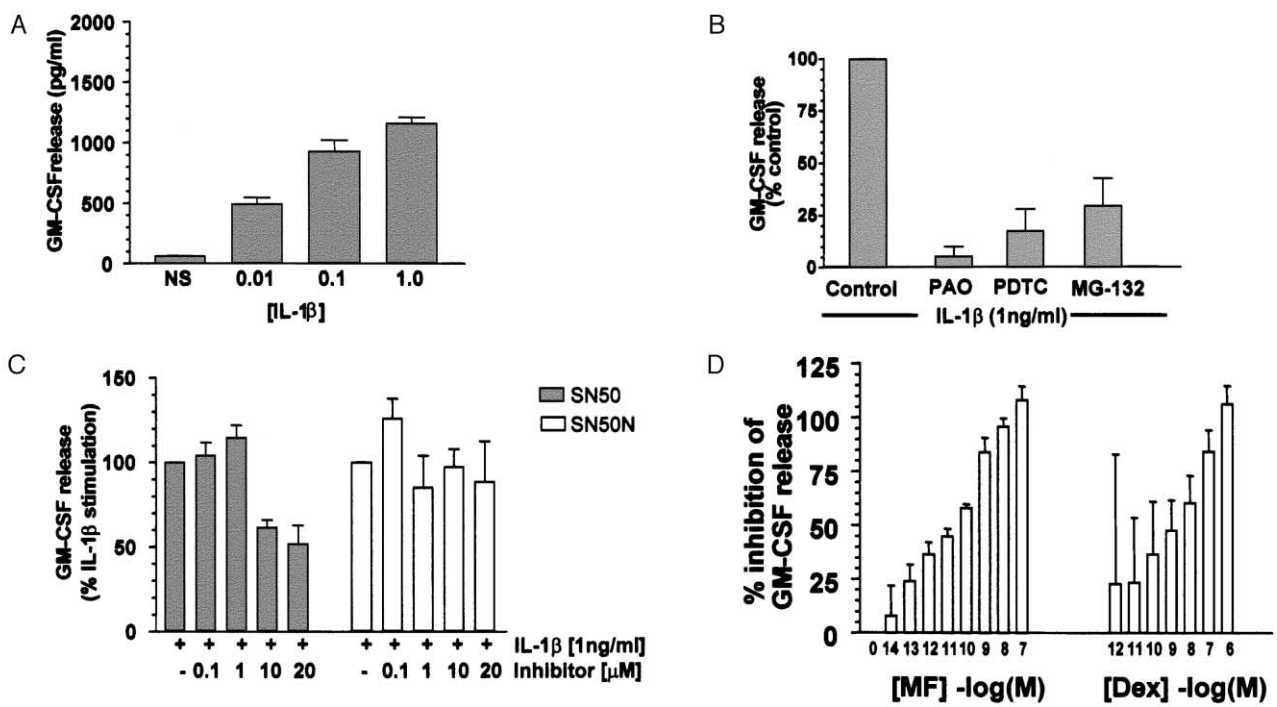

Fig. 1. Role of NF-κB in interleukin (IL)-1β-induced granulocyte/macrophage colony stimulating factor (GM-CSF) release. (A) IL-1β produced a concentration-dependent elevation of GM-CSF release as compared to control unstimulated cells. (B) The effects of phenylarsine oxide (PAO, 10 μM), pyrrolidine dithiocarbamate (PDTC, 1 mM) and the proteasome inhibitor MG-132 (50 μM) on IL-1β (1 ng/ml)-stimulated induction of GM-CSF release at 24 h. (C) Effect of the NF-κB nuclear localisation blocking peptide (SN50) and its control (SN50N) on IL-1β-stimulated GM-CSF release. Results are plotted as the means ± S.E.M. of the percentage of maximal IL-1β-stimulated GM-CSF release in the absence of any drug. $n = 3–7$ for each data set. (D) Concentration-dependent inhibition of IL-1β-stimulated GM-CSF release into the media from cells at 24 h following dexamethasone (Dex) and mometasone furoate (MF) treatment. Results are plotted as the means ± S.E.M. of the percentage of maximal IL-1β-stimulated GM-CSF release in the absence of any drug.

sone furoate ($EC_{50} = 2 \times 10^{-11}$ M) gave a concentration-dependent inhibition of interleukin-1β-stimulated GM-CSF release (Fig. 1D).

3.2. Effect of trichostatin A and 5-azacytidine on interleukin-1β-stimulated GM-CSF release and dexamethasone suppression of interleukin-1β-stimulated GM-CSF release

Interleukin-1β induced both a concentration and time-dependent induction of histone acetylation. Histone acetylation, measured by either activity assays or Western blotting of acetylated histone H4, was increased within 30 min of interleukin-1β (1 ng/ml) stimulation (Fig. 2A). This reached a maximum at 4 h, remained elevated at 8 h and was returned to basal levels by 24 h. Measurement of acetylated histone H4 or cell activity assays at 4 h following stimulation showed a clear concentration-dependent induction that reached a plateau at 1 ng/ml (Fig. 2B).

The response of interleukin-1β-stimulated GM-CSF release to trichostatin A followed a bell-shaped concentration–response curve. Low concentrations caused a concentration-dependent induction of interleukin-1β-induced GM-CSF release with a maximal effect seen at 1 ng/ml (2312 ± 127 vs. 1560 ± 87 pg/ml, $P < 0.05$) (Fig. 2C). At higher concentrations of trichostatin A (100 ng/ml), suppression of GM-CSF release was observed due to gene silencing and perinuclear localisation of chromatin. In addition, inhibition of DNA methylation with 5-azacytidine caused a concentration-dependent induction of interleukin-1β-induced GM-CSF release with a maximal 35% induction seen at 10 μM (755 ± 17 vs. 999 ± 8 pg/ml, $P < 0.01$) (Fig. 2D). 5-Azacytidine (3 μM) markedly enhanced the effect of trichostatin A on interleukin-1β-stimulated GM-CSF release (Fig. 2E).

Histone acetylation status and DNA methylation also affected the ability of glucocorticoids to inhibit interleukin-1β-stimulated GM-CSF release. The ability of mometasone furoate (10^{-11} M) to inhibit interleukin-1β-stimulated GM-CSF release (1.15 ± 0.11 vs. 0.61 ± 0.07 ng/ml) was attenuated by trichostatin A (1.01 ± 0.09 ng/ml) and 5-azacytidine (1.13 ± 0.11 ng/ml) (Fig. 2F). Trichostatin A (10 ng/ml) caused a significant rightward shift in the ability of dexamethasone to inhibit interleukin-1β-stimulated GM-CSF release ($IC_{50} = 1.0 \times 10^{-9}$ vs. 7.8×10^{-8} M, $P < 0.05$). 5-Azacytidine (3 μM) induced a log shift in the ability of dexamethasone to repress inter-

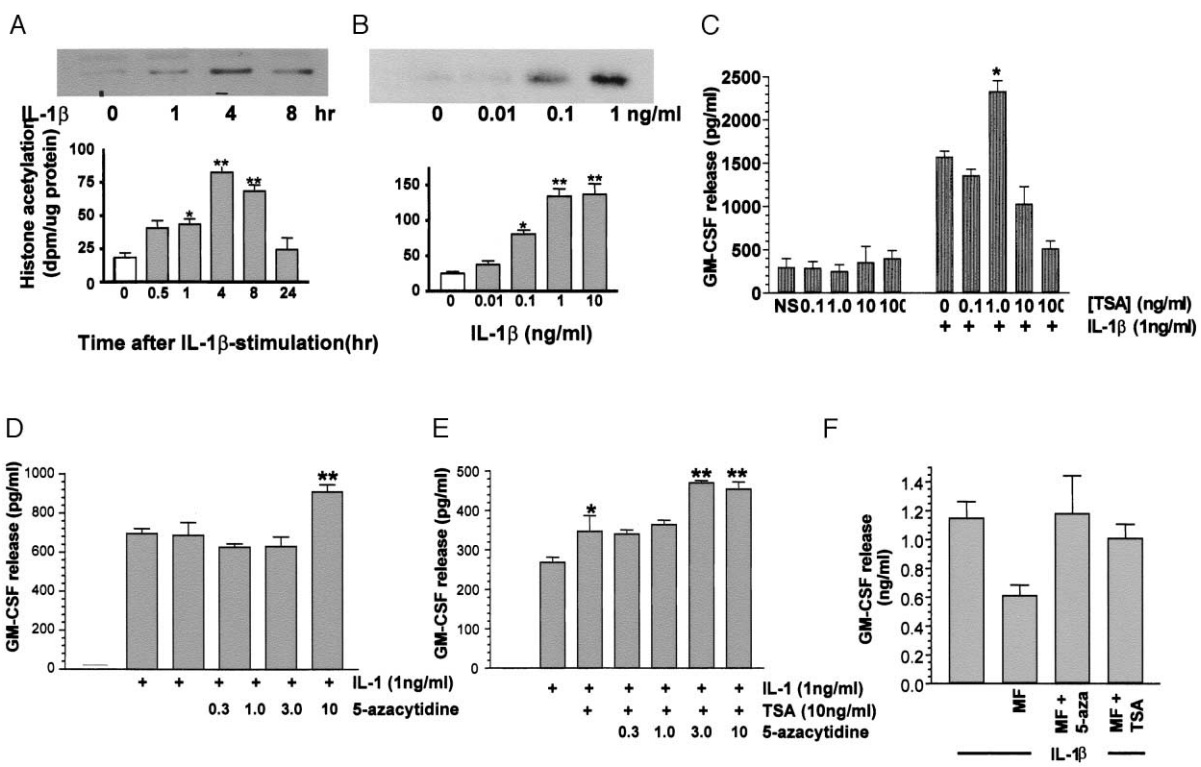

Fig. 2. Concentration-dependent effect of trichostatin A (TSA), a histone deacetylase inhibitor on basal and IL-1β-stimulated GM-CSF release in A549 cells. IL-1β shows a (A) time- and (B) concentration-dependent induction of histone acetylase activity as evidenced by Western blot analysis of whole cell histone H4 acetylation (upper panel) and direct histone acetylase (HAT activity, lower panel). Results are expressed as representative Westerns of four independent experiments or as mean ± S.E.M. of four independent results. (C) Effect of the histone deacetylase inhibitor trichostatin A (TSA) on basal and IL-1β (1 ng/ml)-stimulated GM-CSF release. (D) Effect of the DNA demethylation agent 5-azacytidine (5-aza) on IL-1β-stimulated GM-CSF release. (E) 5-Azacytidine enhances the inductive effect of TSA on IL-1β-stimulated GM-CSF release. (F) The effect of TSA and 5-aza on the ability of 10^{-11} M mometasone furoate (MF) to inhibit IL-1β-stimulated GM-CSF release. Results are plotted as the means ± S.E.M. $n = 4–6$ for each data set except in (F) where results are plotted as the means of three independent experiments.

leukin-1β-stimulated GM-CSF release ($IC_{50} = 2.34 \pm 0.3 \times 10^{-10}$ vs. $1.75 \pm 0.9 \times 10^{-9}$ M, $P < 0.01$). A similar effect was seen with 10 μM 5-azacytidine ($IC_{50} = 2.34 \pm 0.3 \times 10^{-10}$ vs. $0.95 \pm 0.13 \times 10^{-9}$ M, $P < 0.01$). This suggests that acetylation and methylation may co-ordinate to regulate interleukin-1β stimulation of GM-CSF release and to modify glucocorticoid actions.

3.3. CBP modulation of GM-CSF release

Since CBP had been reported to modify NF-κB/GR cross-talk and contains intrinsic histone acetyltransferase activity, we examined the effect of CBP over-expression on interleukin-1β-stimulated GM-CSF release. CBP expression was markedly enhanced, following 18–24 h incubation with pRc/RSVmCBP (data not shown). GM-CSF release following interleukin-1β (1 ng/ml) stimulation was enhanced by over-expression of CBP (2.32 ± 0.18 ng/ml vs. 1.07 ± 0.09 pg/ml, $P < 0.01$) (Fig. 3A). Furthermore, the ability of mometasone furoate (10^{-11} M) to inhibit interleukin-1β-stimulated GM-CSF release was markedly attenuated in these cells (0.46 ± 0.11 vs. 1.03 ± 0.14 ng/ml), whereas a control plasmid had no effect on this inhibition (0.57 ± 0.09 ng/ml) (Fig. 3B). In addition, the mometasone concentration–response curve was shifted to the right following CBP over-expression (pre: EC_{50} of 1.4×10^{-11} M vs. post: EC_{50} of 1.1×10^{-10} M, $P < 0.001$) but not with a control empty vector (Fig. 3C). CBP over-expression did not affect the maximal inhibitory response to mometasone furoate since at doses greater than 10^{-9} M, there was complete inhibition of GM-CSF release in each group.

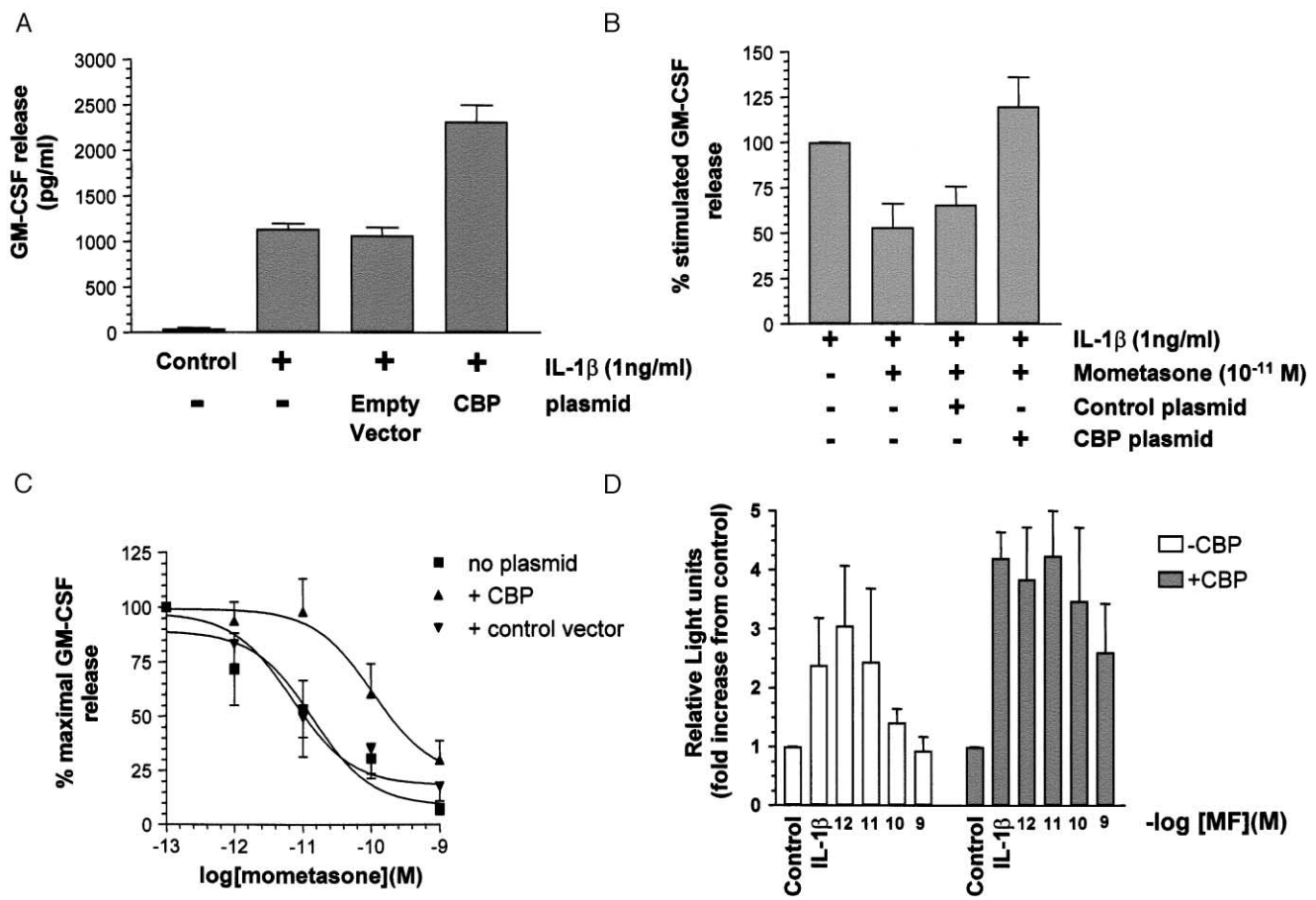

Fig. 3. Effect of CBP expression on inhibition of IL-1β-stimulated GM-CSF release by mometasone furoate. (A) CBP caused a significant ($P < 0.01$) increase in IL-1β-stimulated GM-CSF release after 24 h as compared to that seen in control cells. Transfection of an empty control vector had no effect on IL-1β-stimulated GM-CSF release. (B) The effect of CBP expression on mometasone furoate (10^{-11} M) inhibition of GM-CSF release. Mometasone furoate inhibited IL-1β-stimulated GM-CSF release was markedly attenuated by over-expression of CBP. (C) CBP over-expression (▲) causes a rightward shift in the ability of mometasone furoate (■) to inhibit IL-1β-stimulated GM-CSF release. Transfection of a control empty plasmid (▼) resulted in no significant effect on GM-CSF release. The effect of CBP over-expression was lost at the higher doses of mometasone furoate tested. Results in (B) and (C) are plotted as the means ± S.E.M. of the percentage of maximal IL-1β-stimulated GM-CSF release in the absence of any drug. $n = 4–7$ for each data point. (D) The ability of mometasone furoate (MF) to inhibit IL-1β-stimulated 6× κB luciferase activity was determined in the absence (−CBP, □) and the presence (+CBP, ■) of CBP over-expression. Results are expressed as relative light units/unit β-galactosidase activity. Results are plotted as the means ± S.E.M. $n = 3–6$ for each data point.

3.4. CBP mediation of GM-CSF and κB reporter gene activity

Interleukin-1β-stimulated 6× κB-activated luciferase activity was inhibited in a concentration-dependent manner by mometasone furoate ($EC_{50} = 2.6 \times 10^{-11}$ M) (Fig. 3D). In addition, mometasone furoate also caused a dose-dependent inhibition of basal, non-stimulated, 6× κB-driven luciferase activity ($EC_{50} = 1.0 \times 10^{-11}$ M). There was a greater degree of interleukin-1β (1 ng ml)-stimulated 6× κB luciferase activity in cells over-expressing CBP compared to the lower amount of stimulation seen without CBP over-expression (4.2 ± 0.4-fold increase vs. 2.4 ± 0.8-fold increase). Similar to the results for GM-CSF-luciferase activity, the inhibitory effect of mometasone furoate on 6× κB activity was attenuated at all doses studied following CBP over-expression (Fig. 3D).

3.5. NF-κB and GR can complex with CBP

Cells were incubated for 30 min in the presence of 1 ng/ml of interleukin-1β and mometasone furoate (10^{-9} M) before total cellular proteins were isolated. Anti-CBP, anti-glucocorticoid receptor and anti-p65 immuno-precipitated proteins co-precipitated with p65 as determined by Western blot analysis of the resulting protein-A pellet. The failure of anti-p65 antibodies to precipitate markedly increased amounts of p65 as compared to that precipitated with anti-glucocorticoid receptor antibodies may reflect the relative affinity of the anti-p65 antibody. As a negative control, the ability of p65 to interact with I-κBα or pre-immune serum was examined. There was no association between p65 and I-κBα, presumably because all the I-κBα was degraded after 30 min of interleukin-1β treatment. Pre-immune serum did not immunoprecipitate any of the proteins studied (Fig. 4A). The converse experiment using an anti-p65 or anti-glucocorticoid receptor antibody for immunoprecipitation followed by Western blot analysis with an anti-CBP antibody showed a specific CBP (265 kDa) band along with a weak band in the region of 80 kDa in each lane (Fig. 4B).

4. Discussion

We have confirmed previous reporter gene data (Kochetkova and Shannon, 1996) showing that the interleukin-1β-stimulated increase in GM-CSF release involves NF-κB by the use of selective inhibitors phenylarsine oxide, pyrrolidine dithiocarbamate, and the proteasome inhibitor MG-132. This effect is regulated by changes in histone acetylation and DNA methylation status. Over-expression of the histone acetyltransferase CBP increased the absolute levels of interleukin-1β-stimulated GM-CSF release, enhanced interleukin-1β-stimulated NF-κB activity, and modulated glucocorticoid-mediated inhibition of GM-CSF by mometasone furoate and dexamethasone.

In addition, we have shown that there is an interaction between the p65 subunit of NF-κB and CBP, between p65 and the glucocorticoid receptor, and between the glucocorticoid receptor and CBP after stimulation of the cells with interleukin-1β and mometasone furoate. This may reflect either a direct protein–protein interaction or a complex containing these proteins, and probably others, linked by associated co-factors. The results shown here reflect the central modulatory role of CBP, and its associated factors, in controlling gene transcription, and suggest that modulation of histone acetylase activity could be important in modulating inflammatory processes.

CBP may participate in a transcription complex by interacting with the glucocorticoid receptor- or NF-κB-associated co-factors (Yao et al., 1996; Heery et al., 1997) instead of with the glucocorticoid receptor or NF-κB itself. Although we have shown that the glucocorticoid receptor and p65 may also form a complex with each other, and with CBP, this is not a rule of the requirement for co-

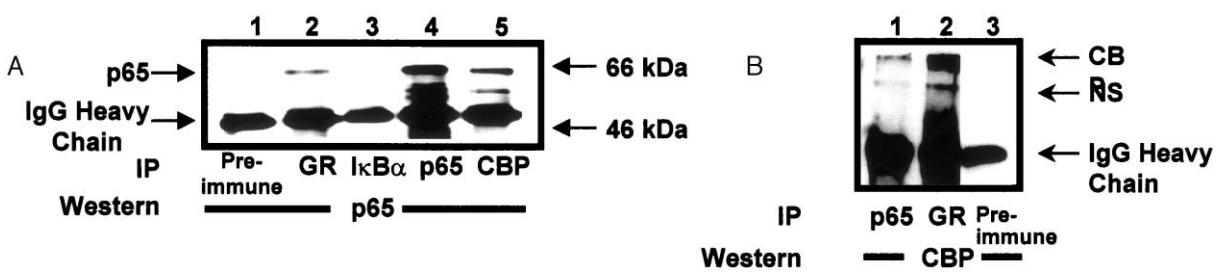

Fig. 4. Interaction of the p65 subunit of NF-κB and the glucocorticoid receptor (GR) with CBP. (A) Western blot analysis of immunoprecipitated p65 complexes. Total cell extracts from IL-1β and MF-treated cells were immunoprecipitated with anti-human GR antibody (lane 2), anti-CBP antibody (lane 5), anti-p65 antibody (lane 4), anti-I-κBα antibody (lane 3) or with pre-immune serum (lane 1) before separation by 10% PAGE and detection of bands by anti-human p65 antibody. The specific p65 band is indicated by an arrow. The IgG heavy chain is detected in all samples and is also arrowed. The results are representative of three independent experiments. (B) Western blot analysis of immunoprecipitated p65 and GR complexes. Total cell extracts were immunoprecipitated with anti-p65 antibody (lane 1), anti-GR antibody (lane 2) or with pre-immune serum (lane 3) before separation by 6% PAGE and detection of bands by anti-human CBP antibody. The specific CBP band is indicated by an arrow. The IgG heavy chain is detected in all samples and is also arrowed. A faint non-specific band (NS) at 80 kDa is detected in the anti-GR and anti-p65 immunoprecipitated lane. The results shown are representative of three independent experiments.

activators or co-repressors being involved in this complex. Indeed, Sheppard et al. (1998) have shown a role for steroid receptor coactivator-1 (SRC-1), as well as CBP, in mediating the interaction between the glucocorticoid receptor and NF-κB. Thus, a multi-component complex may exist involving numerous molecular interactions of varying intensities between a large number of both pro- and anti-inflammatory transcription factors and a number of integrator molecules. The final response, either an increase or decrease in gene transcription, may be acutely sensitive to small changes in either the relative numbers or levels of these transcription factors or in changes in their activation status.

Acetylation of histones produces a loose, less-regulated, chromatin structure that allows transcription factors to bind to nearby promoter sequences, thus, activating gene transcription. The use of histone de-acetylase inhibitors and direct measurement of histone acetylases show that the actions of mometasone furoate and dexamethasone may also be due to a modification of the ability of CBP to regulate histone acetylation (Wolffe, 1997). The fact that histone hyper-acetylation not only attenuates mometasone furoate and dexamethasone inhibition of interleukin-1β-stimulated GM-CSF release but also increases basal levels suggests that histone acetylation and deacetylation are tightly regulated ongoing processes within the cell. This may enable the cell to respond to activation of CBP, or CBP-associated proteins, very rapidly by small changes in the relative activities of either histone acetylases or deactylases and subsequent modification of nucleosome structure. The effects of the DNA demethylation agent 5-azacytidine suggest that the methylation state of DNA may also modulate glucocorticoid receptor actions. These results also reflect previous data showing that the ability of various transcription factors to interact with the basal transcription apparatus, and subsequently modulate transactivation, may depend upon the structure of the associated chromatin/DNA complex (Pennie et al., 1995).

Methylation of cytosines in the cytosine/guanosine (CpG) dinucleotide is generally associated with transcriptional repression in mammalian cells, and recent findings implicate histone deacetylation in methylation-mediated repression. Results in MEL cells using 5-azacytidine and trichostatin A suggest that partial demethylation re-establishes the trichostatin A-inducible state suggesting that in some cases, silencing via methylation of DNA can occur independently of histone deacetylation (Lorincz et al., 2000). Similar results were found in human lung cancer cells (H23, H719 and H1299) whereby the effects of trichostatin A are greatly enhanced in the presence of 5-aza-deoxycytidine (Zhu et al., 2001).

The finding that the methyl-cytosine binding protein MeCP2 binds to histone deacetylases and represses transcription supports a model in which MeCP2 recruits histone deacetylases to methylated DNA resulting in histone deacetylation, chromatin condensation and transcriptional silencing (Ng et al., 1999). The methyl-CpG binding protein MBD2 is targeted to methylated regulatory regions and excludes the acetylated H3 and H4 histones, resulting in localised inactive chromatin. The p16/INK4A gene promoter is inducible by 5-aza-deoxycytidine, but the combination of 5-aza-deoxycytidine and trichostatin A is much more effective. Thus, methyl-CpG binding proteins and histone deacetylases associate and co-operate in repressing p16 expression (Magdinier and Wolffe, 2001). Thus, epigenic regulation including DNA methylation and histone acetylation may play an important role in the regulation of gene expression.

Glucocorticoids exert their anti-inflammatory effects largely by interference with the ability of cytokine-activated pro-inflammatory transcription factors to induce inflammatory gene transcription. This interaction may affect transactivation by the pro-inflammatory transcription factor, association with the integrator molecules, histone acetylation, DNA methylation, and subsequently, activation of RNA polymerase II. The exact contribution of each mechanism may vary between cell types and depend upon the cell stimulus. The central role of histone acetylation and DNA methylation in mediating these actions makes these potentially important targets for future anti-inflammatory drug interventions.

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