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Dexamethasone can stimulate G1-S phase transition in human airway fibroblasts in asthma

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ABSTRACT: Corticosteroids are the first line of therapy for asthma. Whether they alter the progression of airway remodelling in asthma is, as yet, unknown.

To determine whether corticosteroids could alter the fibroblast cell cycle the current authors studied the effect of dexamethasone on cultured airway fibroblasts obtained from nine mild-to-moderate, steroid-naïve asthmatics (forced expiratory volume in one second 78±4% predicted), and seven normal controls. Fibroblasts were cultured from endobronchial biopsies obtained *via* bronchoscopy. Cells were exposed to dexamethasone (10^{-9}–10^{-7} M) and studied at 72 h to determine differences in progression through the cell cycle.

In asthmatic fibroblasts, dexamethasone, at concentrations of 10^{-8}M and 10^{-7}M, nearly doubled the number of cells in the S phase (17.8±3.0% and 18.4±3.1%, respectively) compared with untreated fibroblasts (10.3±1.4%). There was no significant effect in normal control fibroblasts. Dexamethasone induced hyperphosphorylation of the tumour suppressor, retinoblastoma (RB) in asthmatic fibroblasts; fibroblasts from normal controls had significantly less hyperphosphorylation of RB. No difference in protein expression of the CCAAT/enhancer binding protein α between the two groups was detected.

This study suggests that dexamethasone can stimulate G1-S phase cell cycle transition in human airway fibroblasts obtained from asthmatics. Whether this leads to enhanced airway remodelling in some individuals remains to be determined.

KEYWORDS: Asthma, cell cycle, dexamethasone, fibroblast

Airway remodelling in asthma is a pathological process that can eventually lead to fixed airway obstruction. The mechanisms leading to airway remodelling include smooth muscle cell proliferation (both hypertrophy and hyperplasia), goblet cell hyperplasia, and collagen deposition [1]. Airway fibroblasts (and myofibroblasts) are important in mediating many of the changes seen in advanced asthma [2]. Located adjacent to the airway epithelium, fibroblasts produce collagen and also secrete cytokines that may attract inflammatory cells to the airway that can perpetuate the inflammatory and fibrotic process in asthma [3, 4].

Inhaled corticosteroids have been the cornerstone of asthma therapy since the late 1990s [5] and while the anti-inflammatory effects of steroids are well documented, their effects on controlling remodelling are less clear. A report by ROTH *et al.* [6] supports the concept that steroids control inflammation and proliferation in asthma through separate pathways. The group demonstrate that while the loss of the CCAAT/enhancer binding protein α (C/EBPα) in bronchial smooth muscle had no effect on glucocorticoid-mediated inhibition of interleukin-6 release, it completely prevented steroids from inhibiting cell proliferation. The selective loss of C/EBPα from the bronchial smooth muscle of asthmatics (peripheral lymphocytes still expressing C/EBPα) was suggested as being partly responsible for both the enhanced airway smooth muscle proliferation in asthmatics and the failure of steroids to abrogate it. Whether airway fibroblasts from asthmatics exhibit a similarly enhanced proliferative potential or resistance to glucocorticoids is not certain.

The current authors previously examined the effect of corticosteroids on DNA synthesis in fibroblasts obtained from asthmatics [7]. Dexamethasone increased DNA synthesis in cultured airway fibroblasts from asthmatics, an effect that was most prominent in cells from mild asthmatics. Whether this response is beneficial or

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detrimental is not clear; however, it does stand in contrast to the effects of corticosteroids on fibroblasts in other organ systems, where proliferation was either decreased or unchanged [8–10]. To further investigate the effects of corticosteroids on airway fibroblast function in asthma, the present authors also examined the effect of dexamethasone on cell cycle proteins and cell cycle progression in airway fibroblasts cultured from asthmatic individuals. The authors demonstrate that at baseline, untreated fibroblasts from asthmatics have an increased number of cells in the S phase, relative to those from normal controls. In addition, dexamethasone was found to induce hyperphosphorylation of the tumour suppressor retinoblastoma (RB) in fibroblasts from asthmatics and stimulate them to progress through the cell cycle, whereas it had no stimulatory effect on fibroblasts from normal controls.

MATERIALS AND METHODS

Subjects

Nine asthmatic subjects with moderate asthma (forced expiratory volume in one second (FEV_1) $78\pm4\%$ predicted), as defined by the National Asthma Education and Prevention Program, and seven normal controls (FEV_1 $101\pm5\%$ pred) were recruited from the general Denver (CO, USA) community. All asthmatic subjects met diagnostic criteria for asthma [11], exhibiting a methacholine provocative concentration causing a 20% fall in FEV_1 (PC_{20}) <8 mg·mL^{-1} and were only maintained on inhaled β_2-agonists, using on average 1–2 puffs·day^{-1}. All subjects were atopic by clinical history and skin testing to common Colorado aeroallergens (defined as >1 positive skin test). The healthy group had no history of asthma and no bronchial hyperresponsiveness (methacholine PC_{20} >25 mg·mL^{-1}). Exclusion criteria included: 1) use of inhaled or oral corticosteroids, leukotriene modifiers, theophylline, cromolyn and/or nedocromil within the previous 6 weeks; 2) an upper respiratory tract infection within the previous 6 weeks; 3) immunotherapy within the previous 3 months; 4) cigarette use of >5 pack-yrs, and any cigarette use within the past year; and 5) significant nonasthma pulmonary disease or other medical illnesses. Informed consent was obtained from all patients for this institutional, review board, approved protocol.

Bronchoscopy

Subjects underwent bronchoscopy with endobronchial biopsy as previously described [12]. The site of biopsy was randomised to either the right or left lower lobe and five-to-six endobronchial biopsy specimens were taken from the tertiary carinae of the right or left lower lobes, under direct visualisation. Prior to bronchoscopy, spirometry was performed before and after the administration of 0.18 mg of albuterol from a metered dose inhaler and 0.4 mg atropine *via i.v.* Lidocaine (4%) was used to anaesthetise the upper airway, and lidocaine (1%) was applied to the laryngeal area, trachea and orifice of the right lower or left lower lobe bronchi *via* the bronchoscope. Supplemental oxygen was administered throughout the procedure, in addition the heart rate and oxygen saturation were also monitored. The subjects' vital signs were monitored in the laboratory after the procedure and subjects were discharged once FEV_1 was 90% of the pre-bronchoscopy and post-bronchodilator value.

Materials

Dulbecco's minimum essential medium (DMEM), Ipegal, propridium iodide, and RNAse were all obtained from Sigma (St. Louis, MO, USA), Trypsin-EDTA, L-glutamine from Gibco (Grand Island, NY, USA), foetal bovine serum (FBS) from Gemini (Woodland, CA, USA), and polyvinylidene diflouride (PVDF) membrane and ECL-plus from Amersham (Buckinghamshire, UK). Antibodies: α-$p21^{Cip1}$ (c-19) and α-C/EBPα (σχ-61) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), α-$p27^{Kip1}$ and α-retinoblastoma (both mouse monoclonal) from Pharmigen (San Diego, CA, USA), and α-β-actin (mouse monoclonal) from Sigma. Secondary horseradish peroxidise (HRP)-conjugated antibodies: donkey α-mouse, donkey α-rabbit from Jackson Labs (West Grove, NJ, USA).

Fibroblast culture

Fibroblasts were cultured from endobronchial biopsy tissue as previously described [12]. Briefly, biopsy specimens were rinsed and cut into several small pieces (~five–six per biopsy) and placed into wells (two pieces·well^{-1}) of a 24-well tissue culture plate, containing DMEM, supplemented with 10% FBS, streptomycin (100 μg·mL^{-1}), penicillin (10,000 U·mL^{-1}), and gentamicin (100 μg·mL^{-1}). The tissue pieces were incubated at 37°C with 5% carbon dioxide and cultured until cell growth was established, >50% confluency (~8–20 days), the media was changed every 2–3 days. Upon reaching >50% confluency, the tissue pieces were removed and the cells were trypsinised, counted, tested for viability and stained for identification. Additionally, cells were seeded in T25 flasks for subsequent cell passages (Becton Dickinson, Franklin Lakes, NJ, USA). All cells were stained with monoclonal mouse antihuman antibodies against fibroblast antigen (Ab-1; Calbiochem, San Diego, CA, USA), vimentin (DAKO, Carpinteria, CA, USA) and smooth muscle α-actin (DAKO). Of the cells, >99% stained positive for Ab-1 and vimentin and negative for smooth muscle α-actin, confirming them as fibroblasts. Only cells from the first through the third passages were used for experiments. Cells were trypsinised and plated into either 6-well plates (for cell cycle analysis) or T-25 flasks (for protein). Cells were plated in DMEM with 0.5% FBS. Either dexamethasone, at increasing concentrations (10^{-9}–10^{-7} M), or vehicle control was added to the cells at the time they were plated, the cells were studied at 72 h.

Flow cytometry

Cell cycle was determined by flow cytometry in propidium iodide stained cells as previously described [13]. After 72 h in dexamethasone or control, cells were digested with trypsin-EDTA from the culture plates and the trypsin inactivated by addition of DMEM with 10% FBS. Cells were collected by low centrifugation, washed with PBS, recollected by centrifugation, stained with Krishan's solution (propridium iodide, sodium citrate.$2H_20$; Ipegal, Sigma), boiled RNAse and incubated overnight at 4°C. The cells were then analysed in the University of Colorado Health Science Centre's (Denver, CO, USA) Flow cytometry core using a Beckman Epics-XL flow cytometer. Histograms of DNA content were analysed using Modfit LT software to determine fractions of the population in each phase of the cell cycle (G0/G1, S, G2/M).

Western blot analysis

At the same time that cells were harvested for flow cytometry (see above) cells in 100 mm^3 dishes were washed with cold PBS, lysed in radioimmunoassay buffer (PBS, 1% ipegal, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), phenylmethylsulfonyl fluoride (10 $mg \cdot mL^{-1}$), aprotinin (30 $\mu L \cdot mL^{-1}$), and sodium orthovanadate [1 mM]), pelleted at 14,000×*g* and the protein concentration of the supernatant determined by Bradford assay. Equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis under reducing conditions (1% β-mercaptoethanol) using either 14 or 6% gels. The proteins were then transferred to PVDF membrane in 20% methanol. Membrane was blocked in 1% nonfat milk and 0.1% Tween-20, probed with appropriate antibodies (see Materials section), detected with appropriate secondary antibodies conjugated to HRP at 1:10,000 dilution and detected using ECL-plus (Amersham). Protein expression was quantified using National Institutes of Health image 1.63 and expressed as arbitrary density units relative to baseline (cells incubated without dexamethasone) as previously described [14].

Statistical analysis

The percentage of cells in S phase with dexamethasone was compared with the negative control within the asthmatic and control group, respectively, *via* a mixed effects model that evaluated repeated measures with contrasts between each condition and the respective negative control. Contrasts were performed at each concentration of dexamethasone to evaluate concentration differences between treated and untreated fibroblasts within each group and treated fibroblasts between each group. A p-value <0.05 was considered significant. The data are presented as the mean $\pm$ SEM.

RESULTS

Subject characteristics

Subject characteristics are shown in table 1.

Dexamethasone increases the number of asthmatic airway fibroblasts in S phase

Early passaged human airway fibroblasts from either asthmatic or normal individuals were cultured in the presence of increasing doses of dexamethasone for 72 h. Cells were then harvested, stained with propidium iodide, and analysed by flow cytometry. Figure 1 is a representative cell cycle profile of fibroblasts from a normal (a and b) and an asthmatic (c and d) individual. It demonstrates that following exposure to 10^{-7} M dexamethasone the percentage of fibroblasts in S phase from asthmatic subjects increased whereas there was little effect on normal fibroblasts. The aggregate cell cycle data for all experiments at various concentrations of dexamethasone is shown in figure 1 (e and f). Fibroblasts cultured from asthmatics had a significant increase ($p<0.05$) in cells in S phase at both 10^{-8} M (17.8±2.9%) and 10^{-7} M (18.4±3.1%) concentrations of dexamethasone compared with untreated controls (10.3±1.4%). This was associated with a significant decrease in the percentage of cells in G0/G1, but no change in the percentage of cells in G2/M phase. In contrast, fibroblasts from normal controls had no significant increase in cells in the S phase (6.3±1.1%) when exposed to 10^{-8} M (7.9±1.6%) or 10^{-7} M (8.2±1.9%) dexamethasone. In addition, even at baseline (no dexamethasone), there were more asthmatic fibroblasts in the S phase than fibroblasts from normal controls (10.3±1.4 *versus* 6.3±1.1%, $p<0.05$).

TABLE 1 Subject characteristics

	Normal controls	Asthma	p-value
Sex			
Male	4	4	
Female	3	5	
Age yrs	32.4±3.4	32.2±3.7	0.13
Medication	None	β_2-agonists	
FEV_1 L	4.5±0.2	3.1±0.2	0.001
FEV_1 % predicted	101±4	78±4%	0.002

Data presented as n or mean±SEM. FEV_1: forced expiratory volume in one second.

Figure 2 demonstrates the variability in response to dexamethasone among individual subjects. While the baseline S phase population varied in the asthmatic cells there was a consistent increase in cell cycle progression, following exposure to dexamethasone, whereas there was very little effect in the normal controls.

Dexamethasone induces hyperphosphorylation of retinoblastoma in airway fibroblasts from asthmatics

Cell progression through the G1/S transition into S phase requires that the tumour suppressor RB be hyperphosphorylated [15, 16]. Hyperphosphorylation releases the inhibitory effect of RB on the transcription factor E2F which is important for the transactivation of genes required for DNA synthesis. The current study's data indicates that dexamethasone stimulated airway fibroblasts from asthmatics to progress through G0/G1 phase into S phase. Therefore, it should be possible to detect that dexamethasone induced the hyperphosphorylation of RB. The protein was harvested and RB hyperphosphorylation was assessed both in the asthmatic and normal cells. Figure 3 confirms that dexamethasone led to the hyperphosphorylation of RB in airway fibroblasts from asthmatics. While hyperphosphorylation of RB did occur in the cells of some normal controls it was significantly less than in asthmatic cells. This observation is consistent with the flow cytometry data presented above.

Dexamethasone does not induce $p21^{Cip1}$ or $p27^{Kip1}$ expression

Hyperphosphorylation (and inactivation) of RB occurs through the sequential action of two G1 protein complexes, cyclin D-cdk4 and cyclin E-cdk2 [15]. The kinase function of both these cyclin-cyclin dependent kinase (cdk) complexes can be blocked by the cdk inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ [16]. When they bind to the cyclin-cdk complexes they prevent the hyperphosphorylation of RB which causes G1 arrest. The anti-proliferative effects of rapamycin [17] and 3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors (the statins) [18], are thought

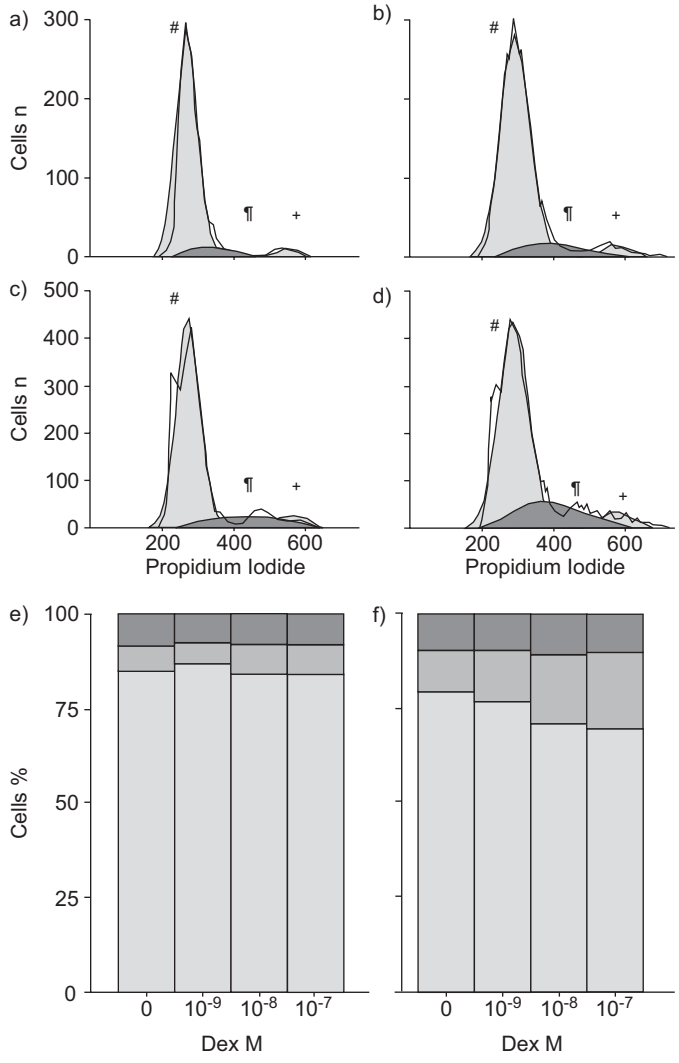

FIGURE 1. Dexamethasone (Dex) stimulates human airway fibroblasts from individuals with asthma into the S phase. a–d) Representative cell cycle profiles of fibroblasts from normal (a and b) and asthmatic (c and d) subjects when exposed to Dex at 10^{-7} M. #: G1 phase; ¶: S phase; +: G2 phase. e and f) represent the aggregate cell cycle data of all the experiments performed in separate patients in normal control group (n=7) and in the asthmatic group (n=9; e and f, respectively). Dex at 10^{-8} and 10^{-7} M significantly increased the number of asthmatic cells in the S phase (■) and decreased the number of asthmatic cells in G0/G1 phase (■, $p<0.05$), but had no effect on cells from normal controls. No significant change in cells in G2/M phase (■) was detected.

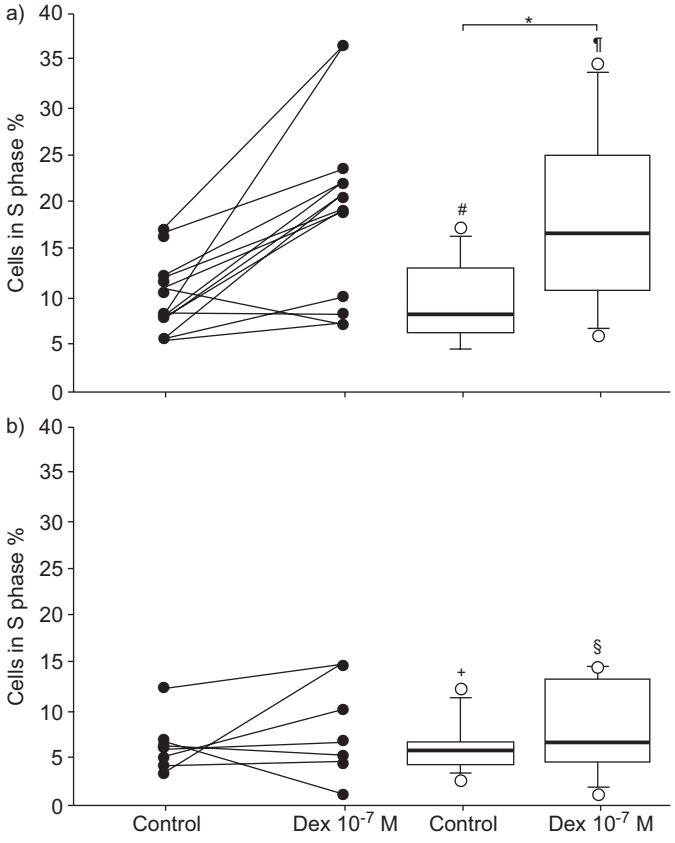

FIGURE 2. Effect of dexamethasone (Dex) on fibroblasts from a) individual asthmatics (n=9) and b) normal controls (n=7). The figure demonstrates the varied responses among fibroblasts from each group in response to Dex 10^{-7} M. *: p-value <0.05 between treated and untreated cells. #: 10.3%; ¶: 18.4%; +: 6.3%; §: 8.2%.

to work, at least in part, through the upregulation of $p21^{Cip1}$ or $p27^{Kip1}$. As a possible explanation for its proliferative effect on airway fibroblasts harvested from asthmatics, protein levels of $p21^{Cip1}$ and $p27^{Kip1}$ were observed to determine whether either was downregulated by dexamethasone. No downregulation of either $p21^{Cip1}$ or $p27^{Kip1}$ in airway fibroblasts was detected from asthmatics after 72 h of dexamethasone exposure. In addition no dexamethasone-induced increase in cyclin D1 (an early G1 protein important in cell cycle progression) was detected. As expected there was some variation between individuals, but as opposed to the consistent effect of dexamethasone on RB hyperphosphorylation there was no observable effect of dexamethasone on $p21^{Cip1}$, $p27^{Kip1}$ or cyclin D1 expression (fig. 4). These results suggest that in the current system dexamethasone did not stimulate cell cycle progression through G1 by downregulating $p21^{Cip1}$ or $p27^{Kip1}$ or by increasing cyclin D1 expression.

A recent report [6] suggested that the loss of the C/EBPα in bronchial smooth muscle from individuals with asthma prevented glucocorticoids from inhibiting proliferation; overexpression of C/EBPα restored glucocorticoid's ability to prevent proliferation. This loss of C/EBPα appeared to be limited to bronchial smooth muscle as normal amounts were detected in peripheral lymphocytes from affected individuals. To determine whether the loss of C/EBPα explained the difference in proliferation between asthmatic and normal fibroblasts, both at baseline and in response to dexamethasone, protein expression in whole cell lysates was examined. The current authors were unable to detect the expression of the 42 kDa isoform of C/EBPα, but only the 30 kDa isoform was found to be present. No significant difference in the expression of this 30 kDa isoform between cell types was identified nor was there any significant effect of dexamethasone on protein expression (fig. 4).

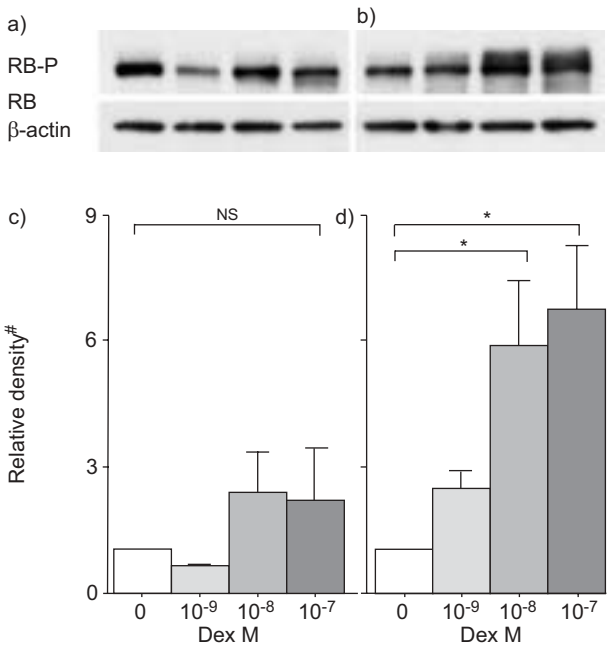

FIGURE 3. a) and b) represent the Western blots for normal (a) and asthma (b) groups. c) and d) represent the relative densitometry results (normalised to β-actin and untreated cells) from five blots in the normal (c) and asthma (d) groups. RB-P: phosphorylated retinoblastoma; RB: retinoblastoma; NS: not significant. *: p-value <0.05;

DISCUSSION

In this study, the authors demonstrated that dexamethasone selectively induces G1-S phase cell cycle transition in airway fibroblasts cultured from asthmatics, while having little effect on fibroblasts from normal controls. This effect is associated with the hyperphosphorylation of RB by dexamethasone.

Within the past 15 yrs the view of asthma has been transformed from a disease of bronchoconstriction to one of chronic, active airway inflammation. Inhaled and systemic corticosteroids have, therefore, replaced bronchodilators as the main arm of therapy. Despite their widespread use, the effect of steroids on airway remodelling in asthmatics is not completely understood. While steroids appear to decrease the influx of eosinophils and other mediators of inflammation their effect on airway epithelial cell and fibroblast function is unclear. Previous studies have suggested that dexamethasone can induce proliferation in fibroblasts obtained from asthmatics, raising the question of a possible deleterious effect on airway remodelling. These current results further suggest that corticosteroids can stimulate airway fibroblast proliferation in asthma.

Little controversy exists regarding the beneficial effects of corticosteroids on controlling the inflammation associated with asthma; less certainty exists on how steroids alter airway remodelling. Since mathematical models suggest that airway wall thickening can account for a large part of the airway hyperreactivity in asthma [19, 20], understanding the role steroids play in this process is of great importance. The difficulty in determining the effects of corticosteroids on airway remodelling in asthma is due, in a large part, to the controversies over how to best define and quantify it. Hyperplasia and hypertrophy of airway smooth muscle, thickening of the basement membrane and production of collagen by fibroblasts have all been identified as markers of airway remodelling in asthma. Predictably steroids have different effects on each process depending on the dose and type of steroid, the model system employed and the measured end-points [21–25].

The current study focused on the effect of steroids upon airway fibroblast proliferation and, therefore, is best compared with studies that address this aspect of airway remodelling. WARSHAMAMA *et al.* [26] demonstrated that dexamethasone increased airway fibroblast proliferation in a human (nonasthmatic) airway fibroblast cell line and also in rat airway fibroblasts; the effect was ameliorated when platelet-derived growth factor isoform AA was blocked. An early study in Swiss 3T3 cells revealed that hydrocortisone increased DNA synthesis, but only in the presence of fibroblast growth factor [8]. However, LEE and BOLS [9] demonstrated that cortisol had no effect on proliferation of rainbow trout fibroblasts [9].

In cells isolated directly from human subjects, DUBE *et al.* [27] demonstrated that proliferation of airway fibroblasts, isolated from subjects with mild-to-moderate asthma, increased 73% above the negative control after exposure to dexamethasone. These data are consistent with the current authors' previous findings, where a 91% increase in DNA synthesis above untreated fibroblasts isolated from subjects with mild asthma was demonstrated [7]. However, in a different model system by SILVESTRI *et al.* [10], fluticasone at concentrations ranging from 0.1–100 nM was found to decrease proliferation and eotaxin expression from fibroblasts cultured from nasal polyps. In addition to the differences in the model system, fluticasone is also 10–100 times more potent than dexamethasone, which may explain the disparate results between these studies [28].

In the current study the authors used a dose of dexamethasone that is within the clinically relevant range and demonstrated that this steroid increased the G1-S phase transition of airway fibroblasts harvested from asthmatic patients. The current authors also demonstrated that even untreated fibroblasts cultured from asthmatic patients had a two-fold increase in the number of cells in S phase at baseline. This suggests important differences exist between asthmatic and normal fibroblasts, in terms of cell cycle regulation, and these differences may contribute to airway remodelling in asthma.

In response to recent reports demonstrating a loss of the transcription factor C/EBPα in bronchial smooth muscle cells from asthmatic patients, total cell lysates from both normal and asthmatic fibroblasts were examined. In contrast to the findings of ROTH *et al.* [6] the current authors were unable to detect the 42 kDa isoform of C/EBPα in protein from either type of fibroblasts. However, equal amounts of the 30 kDa isoform of this protein in fibroblasts from both groups were detected. Production of different C/EBPα isoforms occurs through the use of alternative initiation codons and represents an important form of translational control under different conditions and in different cell types [29]. The 30 kDa isoform is reported to have a lower transactivation potential than the 42

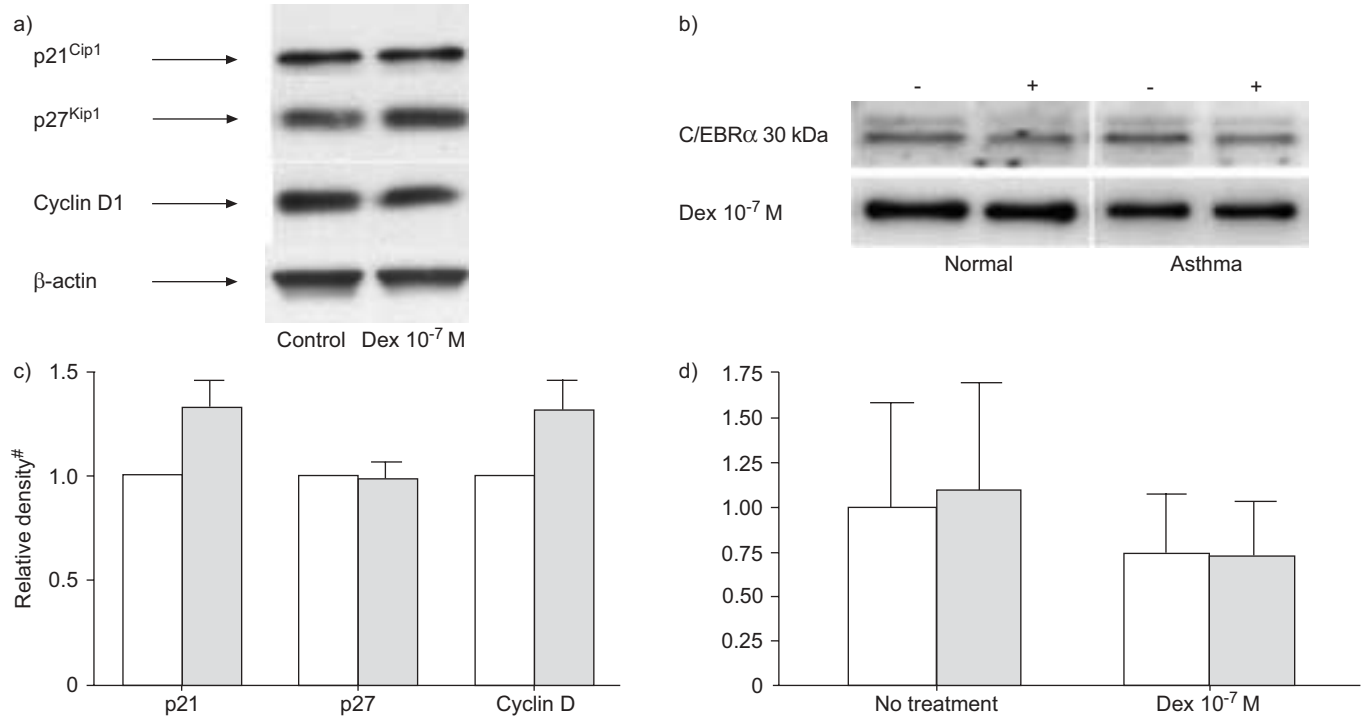

FIGURE 4. a) and b) represent Western blots from a) the asthma group and b) the normal and asthma groups. c) The relative density from seven separate Western blots from the control group with Dex 10^{-7} M (■) and without (□). The differences between Dex treated and untreated asthmatic fibroblasts did not reach statistical significance. d) The relative density from six separate Western blots looking at the expression of the CCAAT enhancer binding protein α (C/EBPα) in fibroblasts from normal subjects (□) and those with asthma (■). No protein expression for the 42 kDa isoform of C/EBPα was detected so only the data for the 30 kDa protein is presented. The differences between the untreated and Dex treated cells was not signficant. #: normalised to control.

kDa isoform, due to the absence of two activating domains; in addition it lacks antimitotic activity in some cells [30, 31]. Whether this difference in isoform expression is due to distinct regulatory functions of C/EBPα between airway fibroblasts and bronchial smooth muscle or is due to differences in experimental conditions is not clear.

The effect of dexamethasone on cell cycle proteins, particularly RB, has been examined by others. Dexamethasone inhibited the hyperphosphorylation of RB and induced G1 arrest in human vascular smooth muscle cells exposed to 10% FBS [32]. In cultured human airway smooth muscle cells, FERNANDES *et al.* [33] demonstrated that dexamethasone could block thrombin-mediated increases in DNA synthesis and did so by blocking the hyperphosphorylation of RB. In contrast, the current authors' experiments demonstrate that dexamethasone (at similar doses) led to the hyperphosphorylation of RB, with an increase in cells progressing through the cell cycle. A few important differences in study design explain these opposing results. First, the current study investigated the effect of dexamethasone on unstimulated (0.5% serum) rather than stimulated (10% FBS or thrombin) cells. This is because the current authors were interested in looking at the potential proliferative effect of dexamethasone on unstimulated fibroblasts, rather than confirming its previously documented antiproliferative effect on cells exposed to growth factors [6, 32, 33]. Secondly the current study investigated earlier passage cells (never using greater than third passage) and finally, the current authors compared cells obtained from patients with asthma to those from normal patients.

An important limitation of this study is that it was not determined whether dexamethasone caused changes in cell number. As a result there are two different conclusions that can be drawn from the data presented. First, that dexamethasone preferentially induced fibroblasts from asthmatics to enter the cell cycle and divide. Secondly, that dexamethasone preferentially induced a prolongation of S phase in fibroblasts from asthmatics causing cells to accumulate there. Further studies will be needed to distinguish between these two possibilities. Another potential limitation is that the current authors studied the effect of dexamethasone on nonsynchronised fibroblasts at a single time point (72 h). This precluded determining the time course during which fibroblasts arrested (synchronised) in G0/G1 would progress from G1 to S phase in response to dexamethasone. Despite these limitations, the marked differences in RB phosphorylation and cell cycle profile between fibroblasts from normal and asthmatic subjects, following exposure to dexamethasone, suggests that the effect of steroids on cells in the airway is not completely predictable and may induce cell cycle progression in fibroblasts from individuals with asthma.

In conclusion, the current data suggests that dexamethasone selectively induces the transition from G1 to S phase of the cell cycle in airway fibroblasts cultured from asthmatics and does so through the hyperphosphorylation of retinoblastoma.

Whether dexamethasone's ability to enhance this transition leads to clinically relevant (and deleterious) effects on airway remodelling, such as enhanced matrix expression by airway fibroblasts, is intriguing but will require further investigation.

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