

Human parainfluenza type 3 virus impairs the efficacy of glucocorticoids to limit allergy-induced pulmonary inflammation in guinea-pigs

William R. FORD*, Alan E. BLAIR*, Rhys L. EVANS*, Elinor JOHN*, Joachim J. BUGERT†, Kenneth J. BROADLEY* and Emma J. KIDD*

*Cardiff School of Pharmacy and Pharmaceutical Sciences, School of Medicine, Cardiff University, Cardiff, U.K.

†Department of Medical Microbiology, School of Medicine, Cardiff University, Cardiff, U.K.

Abstract

Viral exacerbations of allergen-induced pulmonary inflammation in pre-clinical models reportedly reduce the efficacy of glucocorticoids to limit pulmonary inflammation and airways hyper-responsiveness to inhaled spasmogens. However, exacerbations of airway obstruction induced by allergen challenge have not yet been studied. hPIV-3 (human parainfluenza type 3 virus) inoculation of guinea-pigs increased inflammatory cell counts in BAL (bronchoalveolar lavage) fluid and caused hyper-responsiveness to inhaled histamine. Both responses were abolished by treatment with either dexamethasone (20 mg/kg of body weight, subcutaneous, once a day) or fluticasone propionate (a 0.5 mg/ml solution aerosolized and inhaled over 15 min, twice a day). In ovalbumin-sensitized guinea-pigs, allergen (ovalbumin) challenge caused two phases of airway obstruction [measured as changes in sG_{aw} (specific airways conductance) using whole body plethysmography]: an immediate phase lasting between 4 and 6 h and a late phase at about 7 h. The late phase, airway hyper-responsiveness to histamine and inflammatory cell counts in BAL were all significantly reduced by either glucocorticoid. Inoculation of guinea-pigs sensitized to ovalbumin with hPIV-3 transformed the allergen-induced airway obstruction from two transient phases into a single sustained response lasting up to 12 h. This exacerbated airway obstruction and airway hyper-responsiveness to histamine were unaffected by treatment with either glucocorticoid whereas inflammatory cell counts in BAL were only partially inhibited. Virus- or allergen-induced pulmonary inflammation, individually, are glucocorticoid-sensitive, but in combination generate a phenotype where glucocorticoid efficacy is impaired. This suggests that during respiratory virus infection, glucocorticoids might be less effective in limiting pulmonary inflammation associated with asthma.

Key words: dexamethasone, fluticasone propionate, glucocorticoid resistance, parainfluenza type-3 virus, pulmonary inflammation

INTRODUCTION

Respiratory virus infection is a very common cause of asthma exacerbation [1–3] and has been associated with a reduction in the efficacy of glucocorticoid therapy [4,5]. Glucocorticoids are the most effective and widely used anti-inflammatory agents in the treatment of asthma [6]. Clinically, glucocorticoids are recommended for use in all but the most mild forms of asthma [7]. Previously it was recommended that acute exacerbations of asthma be treated by doubling the dose of inhaled corticosteroid [8]. However, a recent Cochrane Review [9] concluded that even doubling the dose of inhaled corticosteroid had no statistically

significant impact on reducing the requirement for rescue oral corticosteroids. Therefore it is important to study the mechanisms and identify treatments that may have the ability to reverse resistance to corticosteroids. Owing to the ethical and logistical difficulties of studying viral exacerbation of human asthma, there is a need to develop animal models that share mechanisms and features with those of asthmatic patients. Murine models have been widely used to study the effects of different respiratory viruses on pulmonary inflammation in sensitized animals [10–15]. However, there are fewer guinea-pig studies of viral exacerbation of allergen-induced pulmonary inflammation [16–20]. The efficacy of glucocorticoids to inhibit the influx of inflammatory cells

Abbreviations: BAL, bronchoalveolar lavage; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FEV₁, forced expiratory volume in 1 s; GR, glucocorticoid receptor; HDAC, histone deacetylase; hPIV-3, human parainfluenza type 3 virus; NF- κ B, nuclear factor κ B; pfu, plaque-forming units; PI3K, phosphoinositide 3-kinase; PIV-1, parainfluenza type-1; RSV, respiratory syncytial virus; RT, reverse transcription; sG_{aw} , specific airways conductance.

Correspondence: Dr William R. Ford (email fordwr@cardiff.ac.uk).

into the airway was reportedly impaired in one of these studies [16] but early and late airways obstruction after allergen challenge has not yet been examined in either mice or guinea-pigs.

We chose to use guinea-pigs where pulmonary inflammation was induced by sensitization and challenge with ovalbumin and airways obstruction monitored as changes in sG_{aw} (specific airways conductance) using dual chamber, whole body plethysmography that allows the non-invasive, repeated measurement of airways obstruction in conscious guinea-pigs [21–25]. Although whole body plethysmography does not provide a detailed measurement of airway function, it does allow repeated assessments of airway conductance to be made in conscious animals. This is of particular importance when late-phase airways obstruction after allergen challenge is being studied owing to inter-individual variability in the timing of the late phase and the fact that a neuronal component would probably be inhibited by the use of a general anaesthetic.

hPIV-3 (human parainfluenza type-3 virus) causes pulmonary inflammation and airways hyper-responsiveness in guinea-pigs as we [26,27] and others [20,28,29] have previously reported. One study in particular reported that pulmonary inflammation induced by bovine parainfluenza type-3 virus was sensitive to beclomethasone treatment but the airway hyper-responsiveness was insensitive [30]. One of the aims of our study was to examine whether viral exacerbation of allergy-induced pulmonary inflammation generated a glucocorticoid-resistant phenotype. For this reason, together with our previous experience [26,27], we elected to use hPIV-3 in the present study.

The aim of the present study was to investigate viral exacerbation of allergy-induced pulmonary inflammation, and its sensitivity to glucocorticoid treatment in guinea-pigs. Our hypothesis is that an interaction between allergy- and virus-induced pulmonary inflammation might lead to the generation of resistance to glucocorticoid treatment.

MATERIALS AND METHODS

Animals

All experimental protocols used male Dunkin–Hartley guinea-pigs (200–250 g; Harlan). Animals were allowed free access to food and water and were housed at room temperature ($22 \pm 2^\circ\text{C}$) with the lighting maintained on a 12 h cycle. This work complied with the guidelines for the care and use of laboratory animals according to the Animals (Scientific Procedures) Act 1986.

Non-invasive measurement of airway function

Changes in sG_{aw} in conscious, spontaneously breathing guinea-pigs were measured using a dual-chamber whole body plethysmograph (PY-5551; Buxco) connected to an LS-20 airway mechanics analyser.

Inoculation with hPIV-3

hPIV-3, obtained from the National Collection of Pathogenic Viruses (Health Protection Agency), was grown in BSC-1 cells (European Collection of Cell Cultures) to a titre of approximately 2.8×10^7 pfu (plaque-forming units). hPIV-3 was harvested and diluted in tissue culture medium [DMEM (Dulbecco's

modified Eagle's medium) supplemented with 1% (v/v) 2 mM L-glutamine, 1% (v/v) non-essential amino acids and 10% (v/v) FBS (fetal bovine serum)] to an approximate titre of 6×10^6 pfu/ml. Guinea-pigs received a bilateral nasal installation (125 μl into each nostril) of hPIV-3 or tissue culture medium obtained from cells grown in the absence of hPIV-3.

RT (reverse transcription)–PCR identification of hPIV-3 in BAL (bronchoalveolar lavage) fluid and lung tissue

RNA was extracted from lung tissue and BAL fluid using PureLink® Viral RNA/DNA kits (Invitrogen) according to manufacturer's instructions. Specific detection and quantification of hPIV-3 was carried out using a Genesig advanced kit for the RT–PCR detection of hPIV-3 (Primerdesign). Amplification and detection of hPIV-3 cDNA was carried out using a Lightcycler (Roche).

Histamine exposure (assessment of hyper-responsiveness)

A baseline reading of sG_{aw} was taken before guinea-pigs were exposed to a histamine aerosol (1 mM, nose only, 20 s) as previously described [19]. Histamine in saline was nebulized using a Wright nebulizer. Immediately after the histamine exposure guinea-pigs were installed into the whole body plethysmograph and sG_{aw} was measured at 0, 5 and 15 min. We have previously determined that exposure to 1 mM histamine does not cause a significant bronchoconstriction in guinea-pigs. Therefore any significant histamine-induced decrease in sG_{aw} value after a treatment protocol indicates the presence of airway hyper-responsiveness to histamine.

Sensitization to ovalbumin

Guinea-pigs were sensitized with two 0.5 ml bilateral intraperitoneal injections of ovalbumin (100 μg) and aluminium hydroxide (100 mg) in PBS administered on days 1 and 5 (Figure 1).

Ovalbumin exposure

Baseline sG_{aw} values were obtained for all guinea-pigs prior to ovalbumin exposure. Sensitized guinea-pigs were placed in a perspex box (40 cm diameter, 15 cm height) and exposed to a nebulized (Wright nebulizer) solution of ovalbumin (10 μg in saline) supplied at a pressure of 20 psi (1 psi = 6.9 kPa) at a flow rate of 0.3 ml/min for 1 h. At the end of the exposure, guinea-pigs were installed in the whole-body plethysmograph and sG_{aw} values were recorded at intervals up to 12 h and again at 24 h after exposure.

BAL and estimation of inflammatory cell counts

The method used has been described in detail previously [23]. Briefly, at the end of a protocol, guinea-pigs were killed by overdose with sodium pentobarbital (Euthatal; intraperitoneal, 400 mg/kg of body weight). The trachea was cannulated and the left lung was clamped. The right lung was lavaged twice with saline (1 ml/100 g of body weight). Total cells in the pooled sample were counted in a Neubauer haemocytometer under light microscopy. Differential cell counts were made after centrifugation

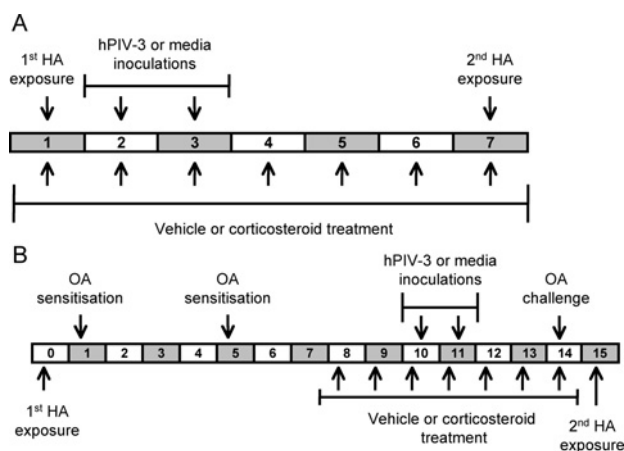


Figure 1 Graphical representation of the experimental protocols
 The protocol used to induce viral pulmonary inflammation in response to hPIV-3 is illustrated in (A) and that used to sensitize guinea-pigs to ovalbumin to assess the impact of hPIV-3 inoculation on allergic pulmonary inflammation in response to challenge with inhaled ovalbumin (B). HA, exposure to inhaled histamine; hPIV-3, inoculation with hPIV-3; media, cell culture medium taken from BSC-1 cells uninfected with hPIV-3; OA, ovalbumin.

(Cytospin, ThermoShandon) of 100 μ l of BAL fluid, transferred onto microscope slides and stained with 0.15% Leishman's solution in methanol.

Experimental protocols

Pulmonary inflammation because of hPIV-3 alone

The experimental protocols are graphically displayed in Figure 1. Guinea-pigs were treated on each of the 7 days with dexamethasone (1 ml/kg of body weight from a 20 mg/ml solution, subcutaneous, once a day), fluticasone propionate (0.5 mg/ml solution inhaled over 15 min, twice a day) or their respective vehicles (saline or saline/DMSO/ethanol, in proportions of 4:3:3 respectively). Airway responses to aerosolized histamine were obtained in all guinea-pigs on day 1 and again on day 7 of the protocol, as previously described. On days 2 and 3 of the protocol, guinea-pigs were inoculated with either hPIV-3 ($\sim 3 \times 10^6$ pfu) or its vehicle (cell culture medium taken from uninfected BSC-1 cells). Following the last histamine exposure, on day 7 of the protocol, the guinea-pigs were killed by an overdose with sodium pentobarbital and BAL performed for total and differential cell counts.

Ovalbumin-induced pulmonary inflammation

The bronchoconstrictor response to inhaled histamine was obtained on the first day of the protocol (day 0, Figure 1B). Subsequently guinea-pigs were sensitized to ovalbumin on days 1 and 5 of the protocol. Guinea-pigs were inoculated with medium obtained from BSC-1 cell culture naïve to hPIV-3 on days 10 and 11 of the protocol. Animals were then treated with dexamethasone (20 mg/kg of body weight, subcutaneous, once a day), fluticasone propionate (0.5 mg/ml, inhalation, twice a day) or their vehicle (saline or saline/DMSO/ethanol, 4:3:3 respectively) for the next 7 days. Animals were challenged with aerosolized ovalbumin on day 14. sG_{aw} was measured over the next 12 h and at 24 h. On day

15, the bronchoconstrictor response to a nose-only exposure of 1 mM histamine was measured. On the same day, after histamine exposure, the animals were killed by an overdose with sodium pentobarbital and BAL performed for total and differential cell counts.

Exacerbation of ovalbumin pulmonary inflammation with hPIV-3

The same protocol as described previously for ovalbumin-induced pulmonary inflammation was followed, except that guinea-pigs were inoculated with medium containing hPIV-3 on days 10 and 11 instead of cell medium alone.

Chemicals and solutions

Aluminum hydroxide, ovalbumin, dexamethasone (water-soluble cyclodextrin complex), fluticasone propionate, histamine diphosphate, DMSO and ethanol were obtained from Sigma-Aldrich. Saline was purchased from Baxter Healthcare. FBS was obtained from Perbio Science UK, and DMEM, L-glutamate and trypsin were from Invitrogen. Euthatal was obtained from Merial Animal Health. Dexamethasone was dissolved in saline and fluticasone propionate in a mixture of saline/DMSO/ethanol in proportions of 4:4:3 respectively.

Data analysis and statistics

All data are presented as means $\pm$ S.E.M. of n guinea-pigs. Changes in sG_{aw} values are expressed as percentage change from baseline measurements (taken immediately before challenge with either histamine or ovalbumin). Cumulative airways obstruction was assessed by calculating the area under the percentage change in sG_{aw} time course (Prism V5.0a for Mac OS; GraphPad Software). Histamine-induced bronchoconstrictions are reported as the mean peak response, measured as percentage change from baseline sG_{aw} , measured over a 15 min interval. Inflammatory cells counted in BAL fluid taken from naïve guinea-pigs (treated with medium taken from BSC-1 cell culture and the vehicle for one or other corticosteroid) have also been reported.

Paired or unpaired Student's t tests were used (Prism V5.0a for Mac OS, GraphPad Software) to identify statistically significant ($P < 0.05$) differences between two means. Where more than two means were compared, ANOVA supported by Bonferroni's post-hoc test for multiple comparisons was used to identify statistically significant ($P < 0.05$) differences among the means.

RESULTS

Airways hyper-responsiveness and inflammation induced by hPIV-3

Inoculation with hPIV-3 of non-sensitized guinea-pigs treated with dexamethasone vehicle ($n = 7$) or fluticasone vehicle ($n = 6$) caused airway hyper-responsiveness to inhaled histamine (Figures 2A and 3A) and an increase in inflammatory cell number in the BAL fluid (Figures 2B and 3B). Treatment with either dexamethasone ($n = 7$; Figure 2A) or fluticasone propionate ($n = 6$, Figure 3A) abolished the exaggerated response to histamine. Compared with naïve guinea-pigs inoculated with medium alone and treated with the vehicle for dexamethasone

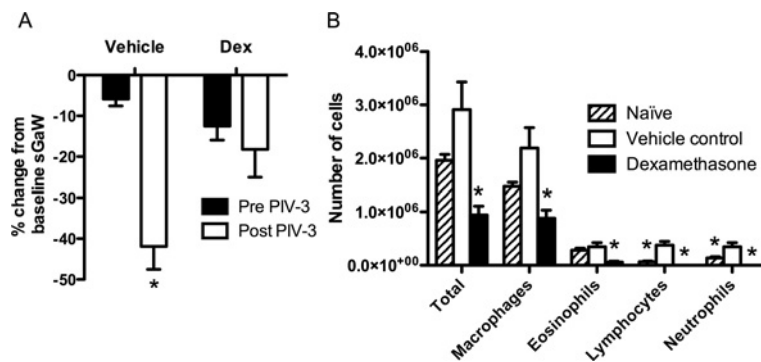


Figure 2 Sensitivity of hPIV-3-induced pulmonary inflammation to treatment with dexamethasone

(A) Peak bronchoconstrictions induced by inhaled histamine in guinea-pigs before (pre PIV-3, closed bars) and after (post PIV-3, open bars) inoculation with hPIV-3. Data are shown for guinea-pigs treated with dexamethasone vehicle (Vehicle, $n = 7$) or dexamethasone (Dex, $n = 7$). * $P < 0.05$ compared with the histamine response elicited prior to inoculation with hPIV-3 (paired Student's t test). (B) Inflammatory cell counts in guinea-pig BAL fluid (taken on day 7 of the protocol after histamine exposure). Data are shown for naïve guinea-pigs (inoculated with culture medium and dexamethasone vehicle but not hPIV-3, hatched bar, $n = 6$) and those inoculated with hPIV-3 and dosed with either dexamethasone vehicle (open bars) or dexamethasone (closed bars). * $P < 0.05$ compared with the dexamethasone vehicle (ANOVA supported by Bonferroni's post-hoc test).

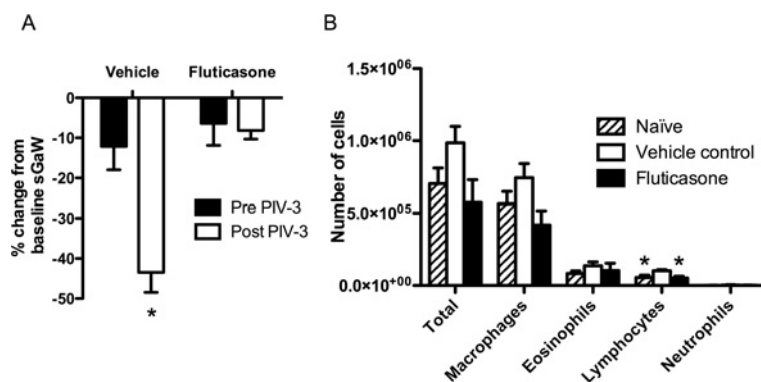


Figure 3 Sensitivity of hPIV-3-induced pulmonary inflammation to treatment with fluticasone propionate

(A) Peak bronchoconstrictions induced by inhaled histamine (1 mM, 20 s nose-only exposure) in guinea-pigs before (pre-PIV-3, closed bars) and after (post PIV-3, open bars) inoculation with hPIV-3. Data are shown for guinea-pigs treated with fluticasone vehicle (Vehicle, $n = 6$) or fluticasone propionate (Fluticasone, $n = 6$). * $P < 0.05$ compared with the histamine response elicited prior to inoculation with hPIV-3 (paired Student's t test). (B) Inflammatory cell counts in guinea-pig BAL fluid (taken on day 7 of the protocol after histamine exposure). Data are shown for naïve guinea-pigs (inoculated with culture medium and fluticasone vehicle but not hPIV-3, hatched bar, $n = 6$) and those inoculated with hPIV-3 and dosed with either fluticasone vehicle (open bars) or fluticasone (closed bars). * $P < 0.05$ compared with fluticasone vehicle (ANOVA supported by Bonferroni's post-hoc test).

($n = 6$) or fluticasone propionate ($n = 7$), PIV-3 inoculation significantly increased lymphocyte and neutrophil numbers in the BAL (Figures 2B and 3B). Dexamethasone treatment significantly decreased the numbers of all inflammatory cell types in the BAL fluid compared with vehicle control guinea-pigs (Figure 2B), whereas only the number of lymphocytes was significantly reduced by fluticasone treatment (Figure 3B).

Effects of dexamethasone on airways responses and inflammation induced by ovalbumin challenge

Early and late airways obstruction

There was a characteristic two-phase response to allergen (ovalbumin) challenge in guinea-pigs sensitized to ovalbumin and treated with dexamethasone vehicle ($n = 6$; Figure 4A–4C). An 'early-phase' of airways obstruction was initiated immediately

after allergen challenge resolving between 4 and 6 h and followed by a distinct 'late-phase' of airways obstruction at approximately 7 h after allergen challenge. Treatment with dexamethasone abolished the 'late-phase' of airways obstruction ($n = 6$; Figures 4A–4C). Peak airways obstruction, measured during the 'early-phase' (0–4 h) was not significantly affected by dexamethasone treatment, whereas it was significantly reduced during the period corresponding to the 'late-phase' (4–24 h; Figure 4B). Cumulative airways obstruction (area under the percentage change in sG_{aw} time course) calculated for the entire 24 h period after allergen challenge, was significantly reduced in sensitized guinea-pigs treated with dexamethasone (Figure 4C). When calculated for the periods corresponding to the 'early-phase' and 'late-phase', dexamethasone significantly reduced cumulative airways obstruction over both phases (Figure 4C).

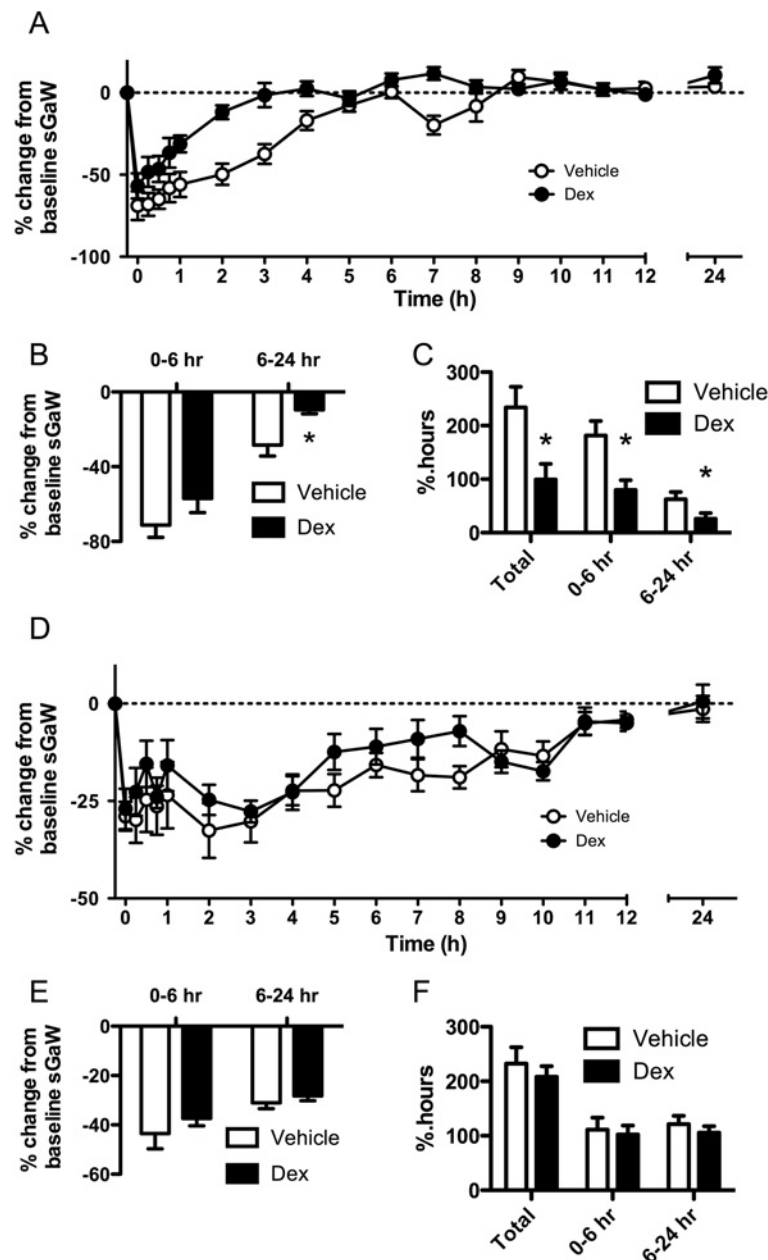


Figure 4 hPIV-3-induced alterations in the time course of pulmonary obstruction after ovalbumin challenge and its sensitivity to dexamethasone

Data are shown for guinea-pigs treated with dexamethasone (Dex, closed circles) or its vehicle (Vehicle, open circles) and inoculated with cell culture medium (**A**, $n=6$ each for dexamethasone and its vehicle) or hPIV-3 (**D**, $n=8$ for both dexamethasone and its vehicle). Peak and cumulative bronchoconstriction measured during periods corresponding to the early (0–6 h) and late phase (6–12 h) are reported for guinea-pigs treated with dexamethasone (Dex, closed bars) or its vehicle (Vehicle, open bars) and inoculated with cell culture medium (**B** and **C** respectively) or hPIV-3 (**E** and **F** respectively).

* $P < 0.05$ compared with dexamethasone vehicle (unpaired Student's t test).

Airways hyper-responsiveness

Prior to allergen challenge, sensitized guinea-pigs exhibited no significant bronchoconstriction in response to histamine exposure (Figure 5A). Exposure to histamine 24 h after allergen challenge caused a significant bronchoconstriction in response to the same concentration of histamine in animals treated with dexamethasone vehicle. Treatment with dexamethasone abolished this

hyper-responsiveness to histamine observed after allergen challenge (Figure 5A).

Inflammatory cell influx

The total number of inflammatory cells counted in BAL fluid taken 24 h after allergen challenge of vehicle-treated animals was significantly increased compared with naïve animals

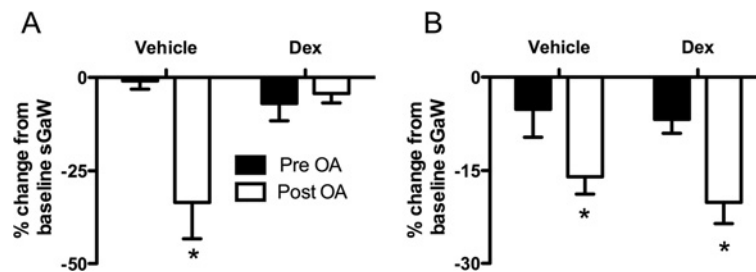


Figure 5 hPIV-3-induced alterations in peak and cumulative airways obstruction after ovalbumin challenge and its sensitivity to dexamethasone

Peak bronchoconstrictions induced by inhaled histamine before sensitization with ovalbumin (Pre OA, closed bars) and after ovalbumin challenge (Post OA, open bars). Data are shown for guinea-pigs treated with dexamethasone (Dex) or its vehicle and inoculated with cell culture medium (A, $n = 6$ for both groups) or hPIV-3 (B, $n = 8$ for both groups). * $P < 0.05$ compared with the histamine response elicited prior to sensitization with ovalbumin (paired Student's t test).

(inoculated with medium, and treated with dexamethasone vehicle but not exposed to inhaled ovalbumin, $n = 6$). This was significantly reduced by treatment with dexamethasone compared with the vehicle control (Figure 6A). Compared with the vehicle control, dexamethasone treatment reduced the numbers of all inflammatory cell types counted in the BAL fluid, except neutrophils. The reduction in inflammatory cells counted in the BAL fluid was such that there were no significant differences between dexamethasone-treated and naïve guinea-pigs.

Effects of dexamethasone on airways responses and inflammation induced by ovalbumin challenge after inoculation with hPIV-3

Early and late airways obstruction

Inoculation of sensitized guinea-pigs with hPIV-3 changed the profile of airways obstruction following allergen challenge in animals treated with dexamethasone vehicle ($n = 8$; Figures 4A and 4D). In the absence of hPIV-3, two distinct phases of airways obstruction were observed (Figures 4A–4C), whereas inoculation with hPIV-3 altered the pattern of response following allergen challenge into a single sustained period of airways obstruction (Figures 4D–4F). Unlike in animals that were inoculated with medium alone, dexamethasone treatment ($n = 8$) had no significant effect on peak or cumulative airways obstruction during the 24 h period following allergen challenge.

Airways hyper-responsiveness

Significant hyper-responsiveness to inhaled histamine was identified in sensitized guinea-pigs inoculated with hPIV-3 and challenged with ovalbumin (Figure 5B). However, unlike sensitized guinea-pigs challenged with ovalbumin and receiving only medium, this hypersensitivity was unaffected by treatment with dexamethasone.

Inflammatory cell influx

Despite a lack of efficacy in reducing airways obstruction and hypersensitivity to histamine, dexamethasone still reduced the numbers of inflammatory cells counted in the BAL fluid obtained 24 h after allergen challenge of guinea-pigs inoculated with hPIV-3 (Figure 6B). Unlike in guinea-pigs treated with dexamethasone

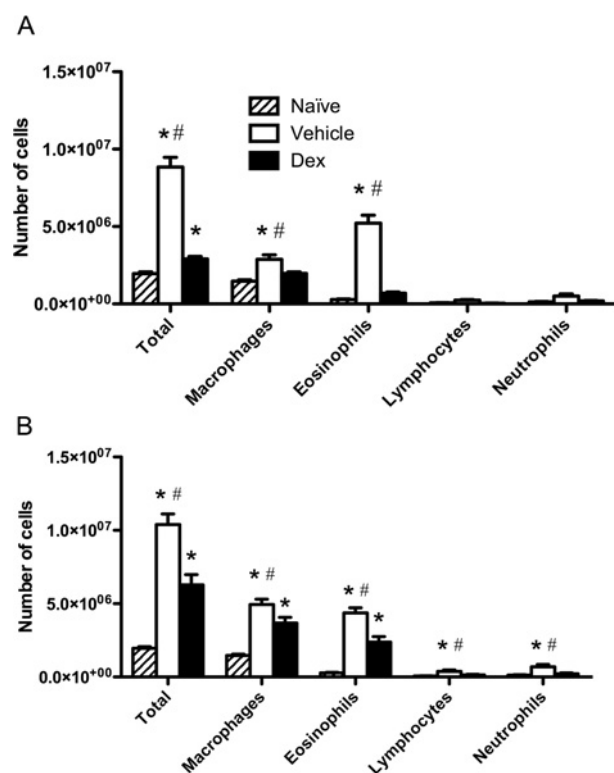


Figure 6 hPIV-3-induced alterations in inflammatory cell influx after ovalbumin challenge and its sensitivity to dexamethasone

Numbers of inflammatory cells in BAL fluid (taken on day 15 of the protocol after histamine exposure) from guinea-pigs treated with dexamethasone (Dex, closed bars) or its vehicle (vehicle, open bars) in guinea-pigs inoculated with cell culture medium (A, $n = 6$ for both groups) or hPIV-3 (B, $n = 8$ for both groups). Also shown are the numbers of inflammatory cells in BAL fluid taken from naïve guinea-pigs (sensitized with ovalbumin, inoculated with culture medium and dexamethasone vehicle but not hPIV-3, hatched bars, $n = 6$). * $P < 0.05$ compared with naïve; # $P < 0.05$ compared with dexamethasone (ANOVA supported by Bonferroni's post-hoc test).

in the absence of hPIV-3 (Figure 6A), the total number of inflammatory cells as well as the numbers of macrophages and eosinophils in the BAL fluid after ovalbumin challenge remained significantly higher than in naïve animals (Figure 6B).

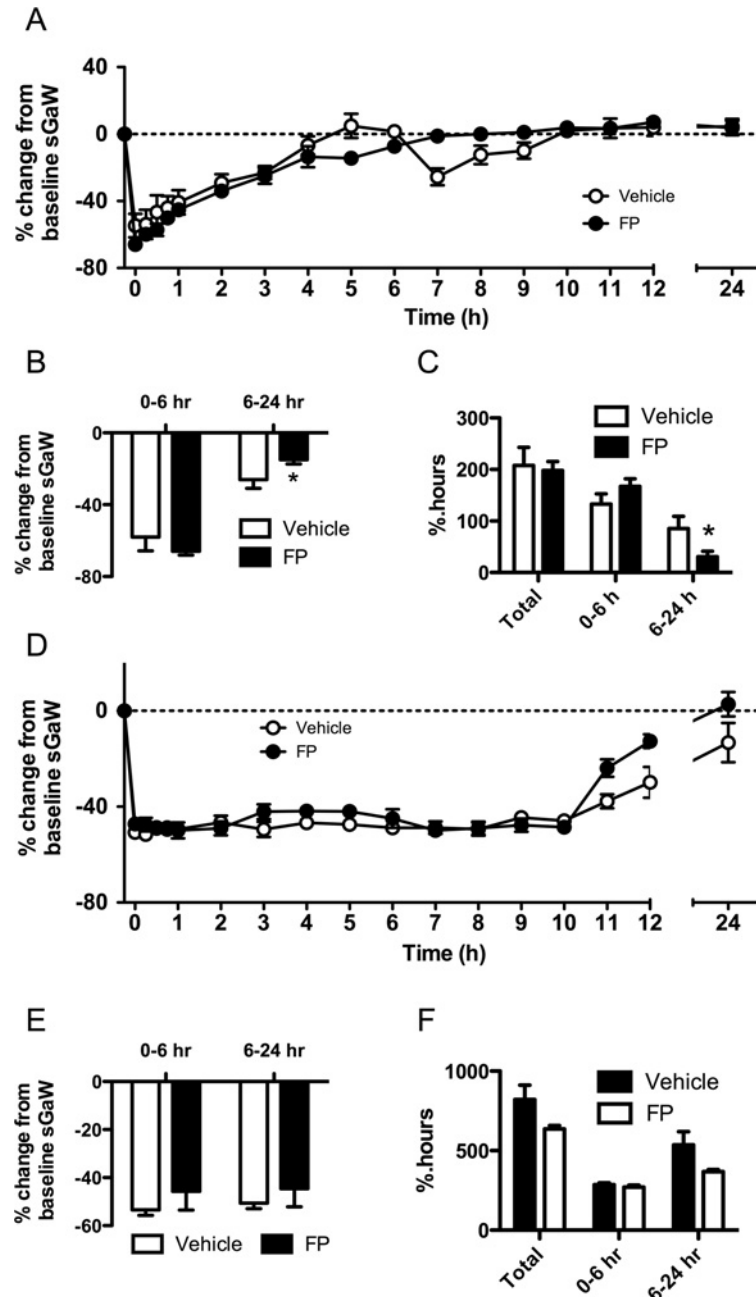


Figure 7 hPIV-3-induced alterations in the time course of pulmonary obstruction after allergen challenge and its sensitivity to fluticasone propionate

Data are shown for guinea-pigs treated with fluticasone propionate (FP; closed circles) or its vehicle (Vehicle; open circles) and inoculated with cell culture medium (A, $n = 6$ for both fluticasone propionate and its vehicle) or hPIV-3 (D, $n = 6$ for both fluticasone propionate and its vehicle). Peak and cumulative bronchoconstriction measured during periods corresponding to the early (0–6 h) and late (6–12 h) phase are guinea-pigs treated with fluticasone propionate (FP; closed bars) or its vehicle (Vehicle; open bars) and inoculated with cell culture medium (B and C respectively) or hPIV-3 (E and F respectively). * $P < 0.05$ compared with fluticasone propionate vehicle (unpaired Student's t test).

Effects of inhaled fluticasone propionate on airways responses to ovalbumin challenge

Early and late airways obstruction

Ovalbumin challenge of sensitized guinea-pigs treated with fluticasone propionate vehicle again produced discrete early and late phases of airways obstruction ($n = 6$; Figures 6A–6C). Like

dexamethasone, treatment of sensitized guinea-pigs with inhaled fluticasone propionate abolished the 'late-phase' airways obstruction ($n = 6$; Figures 7A–7C). Peak airways obstruction measured during the 'early-phase' was not significantly affected by treatment with fluticasone propionate but that measured during the 'late-phase' was significantly reduced compared with the vehicle

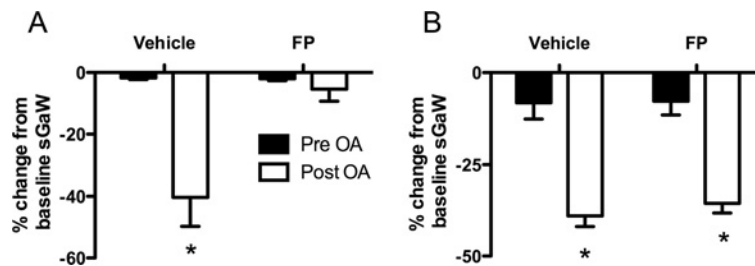


Figure 8 hPIV-3-induced alterations in peak and cumulative airways obstruction after ovalbumin challenge and its sensitivity to fluticasone propionate

Peak bronchoconstrictions induced by inhaled histamine before sensitization with ovalbumin (closed bars) and after ovalbumin challenge (open bars). Data are shown for guinea-pigs treated with fluticasone propionate (FP) or its vehicle and inoculated with cell culture medium (A, $n=6$ for both groups) or hPIV-3 (B, $n=6$ for both groups). * $P < 0.05$ compared with histamine response elicited prior to sensitization with ovalbumin (paired Student's t test).

control (Figure 7B). Unlike dexamethasone treatment, however, fluticasone propionate did not significantly affect the cumulative airways obstruction (area under the percentage change in sG_{aw} time course) calculated for the entire 24 h period after allergen challenge or that calculated for the period corresponding to the 'early-phase' of airways obstruction. The cumulative airways obstruction calculated for the period corresponding to the 'late-phase' of airways obstruction was significantly reduced by treatment with fluticasone propionate (Figure 7C).

Airways hypersensitivity

Hypersensitivity to histamine was again observed in sensitized guinea-pigs treated with fluticasone propionate vehicle following allergen challenge (Figure 8A). This hypersensitivity to histamine was abolished by treatment with fluticasone propionate.

Inflammatory cell influx

The total number of inflammatory cells in the BAL was significantly increased after ovalbumin challenge of vehicle-treated animals compared with naïve guinea-pigs (inoculated with medium and fluticasone vehicle, but not exposed to inhaled ovalbumin, $n=7$). This was significantly reduced by treatment with fluticasone propionate (Figure 9A). Unlike with dexamethasone, only the number of eosinophils was significantly reduced by fluticasone propionate treatment although the levels remained significantly higher than those of naïve guinea-pigs.

Effects of inhaled fluticasone propionate on airways responses to ovalbumin challenge after inoculation with hPIV-3

Early and late airways obstruction

The sustained airways obstruction induced by allergen challenge in sensitized guinea-pigs inoculated with hPIV-3 was insensitive to treatment with fluticasone propionate ($n=6$; Figure 7D) compared with guinea-pigs treated with fluticasone vehicle ($n=6$). Peak airways obstruction during the 24 h period after allergen challenge in sensitized guinea-pigs inoculated with hPIV-3 was unaffected by treatment with fluticasone propionate (Figure 7E). Similarly, cumulative airways obstruction calculated over the 24 h period after allergen exposure was not significantly affected by fluticasone propionate (Figure 7F).

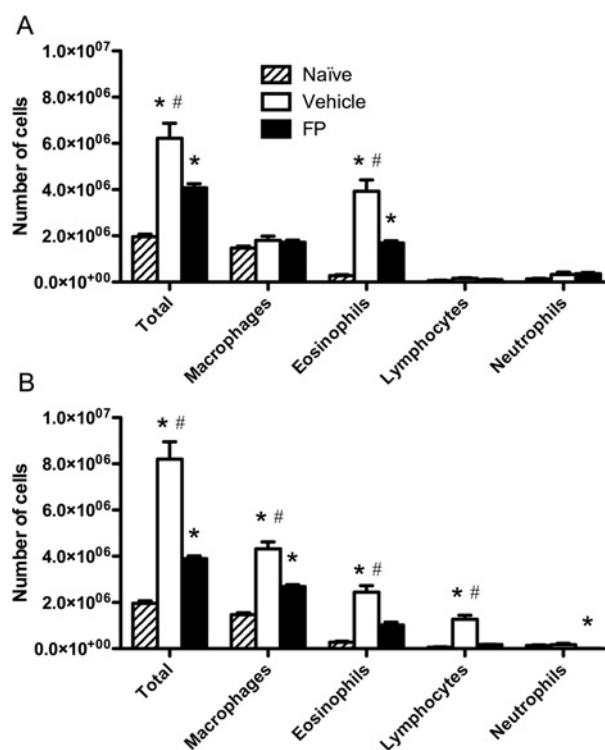


Figure 9 hPIV-3-induced alterations in inflammatory cell influx after ovalbumin challenge and its sensitivity to fluticasone propionate

Numbers of inflammatory cells in BAL fluid (taken on day 15 of the protocol after histamine exposure) from guinea-pigs treated with fluticasone propionate (FP, closed bars) or its vehicle (Vehicle, open bars) and inoculated with cell culture medium (A, $n=6$ for both groups) or hPIV-3 (B, $n=6$ for both groups). Also shown are the numbers of inflammatory cells in BAL fluid taken from naïve guinea-pigs (inoculated with culture medium and fluticasone vehicle but not hPIV-3, hatched bar, $n=6$). * $P < 0.05$ compared with naïve; # $P < 0.05$ compared with fluticasone propionate (ANOVA supported by Bonferroni's post-hoc test).

Airways hypersensitivity

Hyper-responsiveness to inhaled histamine in ovalbumin-sensitized and -challenged guinea-pigs inoculated with hPIV-3 and treated with fluticasone vehicle was insensitive to treatment with fluticasone propionate (Figure 8B).

Inflammatory cell influx

The total number of inflammatory cells counted in the BAL fluid was significantly increased by ovalbumin challenge and inoculation with hPIV-3 in vehicle-treated animals. This was significantly reduced by treatment with fluticasone propionate in sensitized guinea-pigs inoculated with hPIV-3 compared with guinea-pigs treated with fluticasone vehicle (Figure 9B). Macrophage, eosinophil, lymphocyte and neutrophil numbers in the BAL fluid were all significantly reduced in hPIV-3-inoculated sensitized guinea-pigs treated with fluticasone propionate compared with vehicle controls. The total number of inflammatory cells and macrophages remained significantly elevated after fluticasone propionate treatment compared with naïve guinea-pigs (inoculated with medium and fluticasone vehicle, but not exposed to inhaled ovalbumin, $n = 7$). The numbers of eosinophils, lymphocytes and neutrophils in BAL fluid from guinea-pigs treated with fluticasone propionate were reduced to a level not significantly different to that of naïve guinea-pigs.

DISCUSSION

Although the effects of viral inoculation against a background of allergic pulmonary inflammation have been previously reported, this is the first study that we are aware of where the time course of bronchoconstriction in response to allergen challenge and its sensitivity to glucocorticoids has been studied. Pulmonary inflammation and airway hyper-responsiveness to histamine following inoculation with hPIV-3 in non-sensitized guinea-pigs was abolished by treatment with either dexamethasone or fluticasone propionate. Similarly, allergen-induced inflammatory cell influx, hyper-responsiveness to histamine and late-phase allergen-induced airways obstruction was inhibited by treatment with either dexamethasone or fluticasone propionate. The presence of respiratory virus (hPIV-3) during allergen (ovalbumin) challenge of sensitized guinea-pigs profoundly altered the time-course of the airways obstruction after aerosolized allergen challenge such that the classical two-phase response was transformed into a single sustained bronchoconstriction lasting for up to 12 h. Inflammatory cell counts in the BAL fluid were partially reduced by both dexamethasone and fluticasone propionate, neither corticosteroid had a significant effect on airway hyper-responsiveness to histamine or the sustained bronchoconstriction induced by allergen challenge in animals inoculated with hPIV-3.

We are not aware of any previous reports where the profile of airway obstruction following antigen challenge in the presence of respiratory virus has been studied. Indeed, the profile of airway obstruction following allergen challenge is not a commonly used endpoint in either pre-clinical or clinical studies of pulmonary inflammation. In one clinical study where the effect of respiratory tract infection on repeated FEV₁ (forced expiratory volume in 1 s) measurements in asthmatic patients has been studied [31], the authors reported a sustained fall in FEV₁ over several days in asthmatic children infected with a respiratory virus or bacterium. It would be inappropriate to interpret data from that study as directly supporting the clinical relevance of our own findings of

viral-induced alteration in airway obstruction following allergen challenge in guinea-pigs, not least because there was no evidence of allergen provocation in the clinical study [31]. However, it is notable that respiratory infection in a cohort of asthmatic patients was associated with prolonged falls in FEV₁ as well as resistance to various anti-inflammatory agents although glucocorticoids were not tested in that study.

We have identified two pre-clinical studies where the efficacy of corticosteroids in reducing pulmonary inflammation induced by a combination of respiratory virus and allergen was studied [16,32]. In both these studies, one using mice [32] and the other guinea-pigs [16], the anti-inflammatory efficacy of glucocorticoids was impaired. Taken together with our current findings, these studies using different species and different respiratory viruses suggest that resistance to glucocorticoid treatment could be a common feature of allergen-induced pulmonary inflammation in the presence of a respiratory virus.

As the presence of hPIV-3 did not substantively affect the profile of inflammatory cell populations counted in BAL collected 24 h after challenge with allergen, the mechanism of sustained airway obstruction is likely to involve either humoral or neuronal mediators. As we did not measure the levels of humoral (technically difficult in guinea-pigs due to the lack of species-specific reagents) or neuronal mediators, we have no direct evidence to support this hypothesis. Previous studies have reported an increase [16], no change [19], or a decrease [17] in guinea-pig pulmonary inflammatory cells induced by respiratory virus. Although these studies have all used different protocols and viruses, making comparisons difficult, it is notable that hPIV-3 was the virus used where the presence of respiratory virus had no significant effect on allergy-induced inflammatory cells counted in the BAL [19]. However, the findings of that study are not in total agreement with our own as it was also reported that PIV-3 attenuated allergen-induced hyper-responsiveness to MCh (methacholine), whereas hyper-responsiveness to histamine in our study was unaffected by hPIV-3 inoculation.

One theoretical possibility for the corticosteroid resistance we observed with the combination of viral- and allergen-induced pulmonary inflammation, is that as hPIV-3 was administered before ovalbumin challenge this might have led to pharmacokinetic changes that limited access to GRs (glucocorticoid receptors). Indeed, it is known that respiratory viruses increase mucus secretion in guinea-pigs [33], and in theory, this might reduce the bioavailability of inhaled fluticasone propionate. However, such a mechanism would not explain the reduction in efficacy observed for dexamethasone as it was administered systemically. In addition, fluticasone propionate significantly reduced the number of inflammatory cells counted in the BAL fluid of sensitized guinea-pigs exposed to both virus and allergen, providing evidence of a residual anti-inflammatory effect and access to GRs. In addition, we have used very high doses of dexamethasone and fluticasone propionate in our study, which we know from previous dose-ranging studies are in excess of those required to abolish allergen-induced pulmonary inflammation (results not shown). Therefore it is unlikely that the reduced anti-inflammatory efficacy of glucocorticoids is due to an insufficient dose used or reductions in bioavailability

There are a limited number of studies published where the nature of pulmonary inflammation in guinea-pigs after inoculation with PIV-3 has been reported [26,27,29]. The results from our previous study using human PIV-3 were similar to those reported in the current study and were likewise sensitive to treatment with dexamethasone [27]. Folkerts et al. [29] used bovine PIV-3 again with similar results although the effects of corticosteroid treatment were not assessed. Interestingly, the same group examined the interaction between bovine PIV-3 and ovalbumin-induced allergic pulmonary inflammation [20] where a role for tachykinins was identified. Unfortunately, comparison is difficult as their hypothesis was limited to the investigation of hyper-responsiveness in isolated trachea rather than examining inflammatory cell influx and *in vivo* profile of airway obstruction induced by inhaled allergen.

Viruses other than PIV-3 have been used to generate pulmonary inflammation in guinea-pigs. PIV-1 (parainfluenza type-1) has been studied and shown to induce airway hyper-responsiveness and inflammatory cell influx, predominantly neutrophils and eosinophils, into the BAL fluid of guinea-pigs [34]. However, the effect of combining PIV-1 and allergen challenge has not been studied in guinea-pigs. Likewise, inoculation of guinea-pigs with RSV (respiratory syncytial virus) is also reported to induce airway hyper-responsiveness [35]. In this case, an interaction between virus- and allergen-induced pulmonary inflammation has been studied [17]. Unfortunately, neither the time-course of the airway obstruction following allergen challenge nor the sensitivity of the pulmonary inflammation to corticosteroid was assessed.

Reports of how effective glucocorticoids are at limiting allergy-induced pulmonary inflammation in the presence of a respiratory virus are scarce. The only such publication we have been able to identify used human RSV in mice sensitized to ovalbumin [32]. The authors reported that airway hyper-responsiveness and inflammation were resistant to treatment with systemic dexamethasone treatment, in agreement with our findings in guinea-pigs. Unfortunately the profile of airway obstruction following allergen challenge was not studied.

Several mechanisms have been proposed for glucocorticoid insensitivity seen in asthma exacerbations. For example, there may be changes in the binding affinity to the GR. An increased expression of the GR- β receptor has been shown in macrophages [36] and neutrophils [37] of glucocorticoid insensitive subjects. Glucocorticoids do not normally bind to GR- β , but can interact with GR- α , with which they do bind, thereby reducing the binding of glucocorticoids [38]. Another possible mechanism is a failure of glucocorticoids to inhibit the activation of pro-inflammatory transcription factors such as NF- κ B (nuclear factor κ B) and AP-1 (activator protein-1), which regulate the expression of inflammatory cytokines, enzymes and receptors [39]. Glucocorticoids normally reverse this by recruiting HDAC (histone deacetylase)-2 that reverses the histone acetylation induced by NF- κ B and switches off the activated inflammatory genes. Many asthmatics have reduced HDAC-2 function [40]. This decreased HDAC-2 activity may arise indirectly via the PI3K (phosphoinositide 3-kinase/Akt) pathway, which is increased by oxidative stress. Thus a possible target for reversal of glucocorticoid insensitivity is inhibition of PI3Ks. Indeed, we have shown that the

steroid insensitivity induced by PIV-3 inoculation of ovalbumin challenged guinea-pigs as used here can be reversed by narrow spectrum kinase inhibitors [41,42]. Others have also shown that the PI3K inhibitor LY-294002 attenuates allergic inflammation and reverses corticosteroid insensitivity in mice [43,44].

In summary, inflammatory cell influx in the BAL, airway hyper-responsiveness to histamine induced by either hPIV-3 inoculation alone or challenge with ovalbumin in sensitized guinea-pigs were abolished by treatment with systemic dexamethasone or inhaled fluticasone propionate. Additionally, late-phase airways obstruction associated with ovalbumin challenge in sensitized animals was abolished by glucocorticoid treatment. Inoculation of sensitized guinea-pigs with hPIV-3 radically altered the pattern of airways obstruction in response to inhaled allergen from a 'classical' two-phase response to a sustained long-lasting airways obstruction. Neither airways obstruction nor hyper-responsiveness to histamine were reduced by glucocorticoid treatment. Although inflammatory cell influx into the BAL was reduced by treatment with glucocorticoids, this was only partially effective compared with the reductions obtained in the absence of hPIV-3. Therefore our guinea-pig model might provide some valuable insights into the mechanism of interaction between viral- and allergen-induced pulmonary inflammation.

CLINICAL PERSPECTIVES

- We have studied a guinea-pig model of hPIV-3 exacerbation of an ovalbumin allergy-induced pulmonary inflammation. Using this model we have demonstrated that allergy-induced episodic airways obstruction is transformed to a single long-lasting bronchoconstriction that is insensitive to treatment with either dexamethasone or fluticasone propionate.
- The data from our study suggest that the interaction between viral- and allergy-induced pulmonary inflammation may alter bronchial smooth muscle responses in asthmatics such that allergy-induced pulmonary obstructions are of longer duration and are more resistant to glucocorticoid treatment.
- It is accepted that interactions between viral- and allergy-induced pulmonary inflammations are clinically important. Our data suggest that the guinea-pig model would provide a good system for investigating the mechanisms of interaction as well as useful for testing the efficacy of potential treatments.

AUTHOR CONTRIBUTION

An equal contribution to the original idea, study design, analysis and preparation of the paper was made by William Ford, Emma Kidd and Kenneth Broadley. There was an equal contribution to the experimentation made by Alan Blair, Elinor John, Rhys Evans and Joachim Bugert.

FUNDING

This study was supported by Ferring Pharmaceuticals Ltd and RespiVert Ltd as part of contracted research programmes, and from Cardiff University.

REFERENCES

- 1 Beasley, R., Coleman, E. D., Hermon, Y., Holst, P. E., O'Donnell, T. V. and Tobias, M. (1988) Viral respiratory tract infection and exacerbations of asthma in adult patients. *Thorax* **43**, 679–683
- 2 Bardin, P. G., Johnston, S. L. and Pattemore, P. K. (1992) Viruses as precipitants of asthma symptoms. II. Physiology and mechanisms. *Clin. Exp. Allergy* **22**, 809–822
- 3 Johnston, N. W. and Sears, M. R. (2006) Asthma exacerbations. 1: epidemiology. *Thorax* **61**, 722–728
- 4 Vianna, E. O., Westcott, J. and Martin, R. J. (1998) The effects of upper respiratory infection on T-cell proliferation and steroid sensitivity of asthmatics. *J. Allergy Clin. Immunol.* **102**, 592–597
- 5 Reddel, H., Ware, S., Marks, G., Salome, C., Jenkins, C. and Woolcock, A. (1999) Differences between asthma exacerbations and poor asthma control. *Lancet* **353**, 364–369
- 6 Barnes, P. J. (2006) Corticosteroids: the drugs to beat. *Eur. J. Pharmacol.* **533**, 2–14
- 7 British Thoracic Society Scottish Intercollegiate Guidelines Network (2008) British Guideline on the Management of Asthma. *Thorax* **63** (Suppl. 4), iv1–iv121
- 8 Guidelines on Asthma Management. Statement by the British Thoracic Society, The National Asthma Campaign The Royal College of Physicians of London in association with the General Practitioner in Asthma Group, and the Royal College of Paediatrics and Child Health (1997) *Thorax* **52**, S1–S21
- 9 Quon, B. S., Fitzgerald, J. M., Lemiere, C., Shahidi, N. and Ducharme, F. M. (2010) Increased versus stable doses of inhaled corticosteroids for exacerbations of chronic asthma in adults and children. *Cochrane Database Syst. Rev.*, Art. No.: CD007524 DOI: 10.1002/14651858.CD007524.pub3
- 10 Shin, T. S., Lee, B. J., Tae, Y. M., Kim, Y. S., Jeon, S. G., Gho, Y. S., Choi, D. C. and Kim, Y. K. (2010) Role of inducible nitric oxide synthase on the development of virus-associated asthma exacerbation which is dependent on Th1 and Th17 cell responses. *Exp. Mol. Med.* **42**, 721–730
- 11 Chirkova, T., Petukhova, G., Korenkov, D., Naikhin, A. and Rudenko, L. (2008) Immunization with live influenza viruses in an experimental model of allergic bronchial asthma: infection and vaccination. *Influenza Other Respir. Viruses* **2**, 165–174
- 12 Nazir, R., Khanna, M. and Kulshrestha, R. (2008) Time course of pulmonary pathology, cytokine influx and their correlation on augmentation of antigen challenge by influenza A virus infection. *Indian J. Exp. Biol.* **46**, 151–158
- 13 Bartlett, N. W., Walton, R. P., Edwards, M. R., Anisenco, J., Caramori, G., Zhu, J., Glanville, N., Choy, K. J., Jourdan, P., Burnet, J. et al. (2008) Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* **14**, 199–204
- 14 Marsland, B. J., Scanga, C. B., Kopf, M. and Le Gros, G. (2004) Allergic airway inflammation is exacerbated during acute influenza infection and correlates with increased allergen presentation and recruitment of allergen-specific T-helper type 2 cells. *Clin. Exp. Allergy* **34**, 1299–1306
- 15 Lukacs, N. W., Tekkanat, K. K., Berlin, A., Hogaboam, C. M., Miller, A., Evanoff, H., Lincoln, P. and Maassab, H. (2001) Respiratory syncytial virus predisposes mice to augmented allergic airway responses via IL-13-mediated mechanisms. *J. Immunol.* **167**, 1060–1065
- 16 Yamada, K., Elliott, W. M., Hayashi, S., Brattsand, R., Roberts, C., Vitalis, T. Z. and Hogg, J. C. (2000) Latent adenoviral infection modifies the steroid response in allergic lung inflammation. *J. Allergy Clin. Immunol.* **106**, 844–851
- 17 Dakhama, A., Bramley, A. M., Chan, N. G., McKay, K. O., Schellenberg, R. R. and Hegele, R. G. (1999) Effect of respiratory syncytial virus on subsequent allergic sensitization to ovalbumin in guinea-pigs. *Eur. Resp. J.* **13**, 976–982
- 18 Robinson, P. J., Hegele, R. G. and Schellenberg, R. R. (1997) Allergic sensitization increases airway reactivity in guinea pigs with respiratory syncytial virus bronchiolitis. *J. Allergy Clin. Immunol.* **100**, 492–498
- 19 Kudlacz, E. M. and Knippenberg, R. W. (1995) Parainfluenza virus type-3 infection attenuates the respiratory effects of antigen challenge in sensitized guinea pigs. *Inflammation Res.* **44**, 105–110
- 20 Ladenius, A. R., Folkerts, G., van der Linde, H. J. and Nijkamp, F. P. (1995) Potentiation by viral respiratory infection of ovalbumin-induced guinea-pig tracheal hyperresponsiveness: role for tachykinins. *Br. J. Pharmacol.* **115**, 1048–1052
- 21 Evans, R. L., Nials, A. T., Knowles, R. G., Kidd, E. J., Ford, W. R. and Broadley, K. J. (2012) A comparison of antiasthma drugs between acute and chronic ovalbumin-challenged guinea-pig models of asthma. *Pulm. Pharmacol. Ther.* **25**, 453–464
- 22 Smith, N. and Broadley, K. J. (2010) Adenosine receptor subtypes in airways responses of sensitized guinea-pigs to inhaled ovalbumin. *Pulm. Pharmacol. Ther.* **23**, 355–364
- 23 Smith, N. and Broadley, K. J. (2007) Optimisation of the sensitisation conditions for an ovalbumin challenge model of asthma. *Int. Immunopharmacol.* **7**, 183–190
- 24 Toward, T. J. and Broadley, K. J. (2004) Early and late bronchoconstrictions, airway hyper-reactivity, leucocyte influx and lung histamine and nitric oxide after inhaled antigen: effects of dexamethasone and rolipram. *Clin. Exp. Allergy* **34**, 91–102
- 25 Toward, T. J. and Broadley, K. J. (2002) Airway function, oedema, cell infiltration and nitric oxide generation in conscious ozone-exposed guinea-pigs: effects of dexamethasone and rolipram. *Br. J. Pharmacol.* **136**, 735–745
- 26 Broadley, K. J., Blair, A. E., Kidd, E. J., Bugert, J. J. and Ford, W. R. (2010) Bradykinin-induced lung inflammation and bronchoconstriction: role in parainfluenza-3 virus-induced inflammation and airway hyperreactivity. *Pharmacol. Exp. Ther.* **335**, 681–692
- 27 Toward, T. J., Johnson, F. J., Boulton, J. E. and Maillard, J. Y. (2005) Airway function and reactivity, leukocyte influx and nitric oxide after inoculation with parainfluenza-3 virus: effects of dexamethasone or rolipram. *Int. Immunopharmacol.* **5**, 771–782
- 28 Nijkamp, F. P. and Folkerts, G. (1995) Nitric oxide and bronchial hyperresponsiveness. *Arch. Int. Pharmacodyn. Ther.* **329**, 81–96
- 29 Folkerts, G., van der Linde, H. J., Omini, C. and Nijkamp, F. P. (1993) Virus-induced airway inflammation and hyperresponsiveness in the guinea-pig is inhibited by levodropropizine. *Naunyn Schmiedeberg's Arch. Pharmacol.* **348**, 213–219
- 30 Leusink-Muis, A., Ten Broeke, R., Folkerts, G., De Clerck, F. and Nijkamp, F. P. (1999) Betamethasone prevents virus-induced airway inflammation but not airway hyperresponsiveness in guinea pigs. *Clin. Exp. Allergy* **29** (Suppl. 2), 82–85
- 31 Kondo, S., Ito, M., Saito, M., Sugimori, M. and Watanabe, H. (1994) Progressive bronchial obstruction during the acute stage of respiratory tract infection in asthmatic children. *Chest* **106**, 100–104
- 32 Chi, X. Y., Jiang, S. J., Wang, J. and Wang, J. P. (2011) Effect of glucocorticoid in mice of asthma induced by ovalbumin sensitisation and RSV infection. *Asian Pac. J. Allergy Immunol.* **29**, 176–180
- 33 Folkerts, G., Verheyen, A. and Nijkamp, F. P. (1992) Viral infection in guinea pigs induces a sustained non-specific airway hyperresponsiveness and morphological changes of the respiratory tract. *Eur. J. Pharmacol.* **228**, 121–130
- 34 Nie, Z., Scott, G. D., Weis, P. D., Itakura, A., Fryer, A. D. and Jacoby, D. B. (2011) Role of TNF- α in virus-induced airway hyperresponsiveness and neuronal M2 muscarinic receptor dysfunction. *Br. J. Pharmacol.* **164**, 444–452
- 35 Tan, Y., Yang, T., Liu, S., Liu, H., Xiang, Y., Qu, F., Li, H. and Qin, X. (2008) Infection with respiratory syncytial virus alters peptidergic innervation in the lower airways of guinea-pigs. *Exp. Physiol.* **93**, 1284–1291

- 36 Goleva, E., Li, L. B., Eves, P. T., Strand, M. J., Martin, R. J. and Leung, D. Y. (2006) Increased glucocorticoid receptor β alters steroid response in glucocorticoid-insensitive asthma. *Am. J. Respir. Crit. Care Med.* **173**, 607–616
- 37 Strickland, I., Kisich, K., Hauk, P. J., Vottero, A., Chrousos, G. P., Klemm, D. J. and Leung, D. Y. (2001) High constitutive glucocorticoid receptor β in human neutrophils enables them to reduce their spontaneous rate of cell death in response to corticosteroids. *J. Exp. Med.* **193**, 585–593
- 38 Oakley, R. H., Jewell, C. M., Yudit, M. R., Bofetiado, D. M. and Cidlowski, J. A. (1999) The dominant negative activity of the human glucocorticoid receptor β isoform. Specificity and mechanisms of action. *J. Biol. Chem.* **274**, 27857–27866
- 39 Barnes, P. J. (2006) Corticosteroid effects on cell signalling. *Eur. Respir. J.* **27**, 413–426
- 40 Ito, K., Chung, K. F. and Adcock, I. M. (2006) Update on glucocorticoid action and resistance. *J. Allergy Clin. Immunol.* **117**, 522–543
- 41 Broadley, K. J., Herbert, A. A., Evans, R. L., Cass, L., Rapeport, G., Strong, P., Kidd, E. J. and Ford, W. R. (2011) Inhibition of steroid resistant viral exacerbations of ovalbumin-induced allergic airways responses in guinea-pigs by a narrow spectrum kinase inhibitor, RV568. *Am. J. Respir. Crit. Care Med.* **183**, A6209
- 42 Broadley, K. J., Herbert, A. A., Evans, R. L., Cass, L., Strong, P., Rapeport, G., Ford, W. R. and Kidd, E. J. (2011) RV1088, a narrow spectrum kinase inhibitor, inhibits steroid resistant viral exacerbations of ovalbumin-induced allergic airways responses in guinea-pigs. *Am. J. Respir. Crit. Care Med.* **183**, A1303
- 43 Barthel, A. and Klotz, L. O. (2005) Phosphoinositide 3-kinase signaling in the cellular response to oxidative stress. *Biol. Chem.* **386**, 207–216
- 44 Mercado, N., To, Y., Ito, K. and Barnes, P. J. (2011) Nortriptyline reverses corticosteroid insensitivity by inhibition of phosphoinositide-3-kinase- δ . *J. Pharmacol. Exp. Ther.* **337**, 465–470

Received 12 March 2013/29 April 2013; accepted 17 May 2013

Published as Immediate Publication 17 May 2013, doi: 10.1042/CS20130130

Copyright of Clinical Science is the property of Portland Press Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.