

Inhaled dexamethasone differentially attenuates disease relapse and established allergic asthma in mice

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Received 13 December 2002; accepted with revision 9 September 2003

Abstract

Inhaled glucocorticoids are effective in patients with chronic allergic asthma. We examined the effects of inhaled glucocorticoids on relapse (allergen challenge after disease remission) and established/overt allergic asthma (repeated allergen challenge in weekly intervals) in mice to establish a reference standard for novel treatments. BALB/c mice were treated before relapse or during overt disease with 1 h of nebulized PBS or 10 mg% dexamethasone twice daily for 5 days. Dexamethasone eliminated airway hyperresponsiveness before relapse and during overt disease. They more efficiently reduced airway inflammation, mucus production, and OVA-specific IgG1 and IgE during relapse compared to overt disease. However, during overt disease, parenchymal inflammatory infiltrates were more effectively eliminated compared to relapse, suggesting that activated infiltrating leukocytes have increased sensitivity to steroids. These data demonstrate that inhaled corticosteroids attenuate relapse and overt disease differentially and suggest that both airway and parenchymal inflammation need to be evaluated for treatment efficacy.

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Keywords: Allergy; Asthma; Mice; Overt; Relapse; Dexamethasone; Glucocorticoids; Treatment; Mouse models

Introduction

Allergic asthma is characterized by episodes of reversible airway obstruction, airway hyperresponsiveness, airway lung inflammation, increased mucus production, and high levels of IgE [1–3]. When patients with allergic asthma are exposed to seasonal allergens or have intermittent contact, they develop disease exacerbations or relapses. However, ubiquitous or chronic allergen exposure leads to a chronic nonremitting form of disease. Though several animal models of allergic asthma are used to study disease pathogenesis and develop novel treatments, many have not been oriented toward treating disease relapses and overt or ongoing allergic asthma. Previously, we established mouse models resembling these clinically relevant phases of disease [4] and discovered that these disease phases differed from initiation of acute disease, suggesting that they may require alternative therapeutic approaches.

One of the most effective treatments for patients with allergic asthma and particularly for those with chronic disease are glucocorticoids [5–7]. Glucocorticoids have a myriad of effects initiated by binding to cytosolic glucocorticoid receptors, translocating to the nucleus, and altering the regulation of inflammatory cytokine gene expression by binding to glucocorticoid-responsiveness gene elements [6,8]. In allergic asthma, this results in the inhibition of macrophages, T-lymphocytes, eosinophils, and epithelial cells [9], reduced numbers of airway mast cells [9–11], and reduced numbers of circulating and airway eosinophils [10,11]. Furthermore, glucocorticoids inhibit airway mucus secretion [12], reduce goblet-cell hyperplasia [10,13], and upon extended inhalation, reduce histamine- and methacholine-induced airway responsiveness [13,14]. Though inhaled steroids have a beneficial therapeutic effect in established and in particular, chronic allergic asthma, adverse effects limit their usefulness and, thus, new therapeutic approaches are necessary.

Corticosteroid therapy has also been shown to influence allergic asthma in animal models [15–17]. However, the effects are often tested on the initial prevention of lung infiltration in sensitized animals and do not investigate the

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effect of infiltration in animals recuperated from disease [15]. Additionally, several studies have administered dexamethasone (DEX) intraperitoneally rather than by aerosol [15]. The effects of DEX have been explored in a schistosoma model [17], and in studies focused on airway remodeling [18–20]. Few studies have focused on interfering with airway inflammation, mucus hypersecretion, serum and bronchoalveolar lavage fluid (BAL) OVA-specific IgE/IgG1, and airway hyperreactivity in established disease. The aims of this investigation were to assess the effects of DEX during relapse and overt disease. Specifically, we sought to determine whether disease phases responded differently to inhaled steroids and whether DEX could be utilized as a reference standard in these models for comparison in future studies with new pharmacological treatments for allergic asthma.

Materials and methods

Mice

Six- to eight-week-old BALB/c mice were maintained in the VIRCC facility and provided OVA-free food and water ad libitum. All experiments complied with the requirements of the Animal Care Committee of the University of Vienna.

Immunization

Male BALB/c mice were immunized with 25 µg of OVA (Sigma Chemical Co., St. Louis, MO) or PBS in 2 mg of alum intraperitoneally on day 0 and day 5. Mice were challenged 1 week later with nebulized 1% OVA in PBS in a Plexiglas chamber by a KLAVA ultrasonic nebulizer for 60 min twice daily on two consecutive days (day 12 and 13) to induce acute onset disease. To induce disease relapse, we rested mice following acute disease and rechallenged with aerosolized OVA on days 41 and 42. Overt disease was generated by treating mice following acute disease with weekly aerosol challenges, 60 min twice daily, on days 20 and 27.

Treatment

Dexamethasone phosphate (Sigma, 10 mg% solution in PBS) or PBS was administered by ultrasonic nebulizer twice daily on days 38–42 to prevent relapse and on days 23–27 to inhibit overt disease. Treatment was administered 1 h before OVA aerosol on days 41 and 42 for relapse and on day 27 for overt disease. Mice were evaluated for treatment effects on days 43 and 48 for relapse and on days 28 and 29 for overt disease.

Bronchoalveolar lavage

The tracheas of lethally anesthetized mice were cannulated and lavaged with three 0.8-ml aliquots of PBS. The

BAL was centrifuged, and cell pellets were resuspended in PBS. Total leukocytes were counted with a hemocytometer. Cytospin slides were made and stained with May-Grünwald-Giemsa to determine the cell differential.

Lung inflammation and mucus production

Following BAL, the pulmonary arteries and tracheas were perfused with formalin. Paraffin-embedded lung sections of 4 µm were stained with Hematoxylin and Eosin (H&E) for morphological evaluation and with Periodic-Acid-Schiff stain (PAS) for mucopolysaccharide staining. The three most densely infiltrated areas in each H&E section were evaluated using an ocular grid with 100 small squares (each small square area = 5929 µm²). Percentages of inflammation area were calculated by counting the cells in the infiltrates within the overlaying grid, divided by the total area under the grid, excluding areas including vascular and airway lumina.

Determination of airway responsiveness

We measured airway hyperresponsiveness in conscious, unrestrained mice by whole-body plethysmography (Buxco Electronics Ltd., Sharon, CT) [4]. PBS, followed by increasing concentrations of methacholine (Sigma), were nebulized for 3 min and lung function was recorded and calculated as enhanced pause (Penh), which is a dimensionless unit that correlates with pulmonary resistance.

Serum and BAL immunoglobulin

For the measurement of OVA-specific IgG1, ELISA plates were coated with OVA at 10 µg/ml overnight at 4°C. The plates were washed and blocked with 2% BSA–0.05% Tween 20 for 2 h at 25°C. Titrated sera or BAL fluid were incubated for 2 h at room temperature. After washing, biotinylated anti-IgG1 detection mAb was added and plates were incubated for 1 h. Europium–streptavidin was added to each well after the plates were washed. One hundred microliters of enhancement solution was added (DELFA, Wallac Oy, Turku, Finland) and Eu³⁺ release was measured by fluorometry at 340-nm excitation and 614-nm emission. Pooled samples from mice with acute disease were used to generate the standard curves. In similar assays, OVA-specific IgE titers were tested by coating plates with antimouse IgE (LO-ME-3; Serotec, UK) and detected with biotinylated-OVA.

Statistical analysis

Groups were compared by unpaired Student's *t* test. Values of *P* were considered significant at <0.05. Results for all measurements are expressed as the mean ± SEM. For airway hyperresponsiveness, we compared the means of

summed Penh for all concentrations of methacholine. Unpaired Student's *t* tests were used to analyze differences between PBS- and DEX-treated groups and treated groups compared to alum alone controls. $P < 0.05$ was considered significant.

Results

Evaluation of DEX treatment on lung inflammation

To determine whether there were differences in lung inflammation between mice treated with DEX before disease relapse or during overt disease, BAL cytology was evaluated. Total BAL cell counts (TBC), which reflect the intensity of airway inflammation, were reduced by 55% when DEX was administered before disease relapse induction compared to PBS controls (Fig. 1a). Remarkably, inhaled DEX did not affect TBC during overt disease (Fig. 1b). DEX treatment before relapse significantly prevented eosinophil infiltration into the airways by 86%, and reduced macrophages and lymphocytes, by 39% and 56%, respectively (Fig. 1a). DEX increased neutrophil infiltration into the lungs by 76% compared to PBS-treated controls (Fig. 1a). However, neutrophils only comprised approxi-

mately 1% of the cellular infiltrate in the airways. During overt disease, inhaled DEX inhibited eosinophil infiltration by approximately 60% compared to PBS controls without significantly affecting the numbers of other leukocytes within the BAL (Fig. 1b). These data demonstrate that eosinophil infiltration in the airways is inhibited during relapse and overt disease, but that overall inflammation can be reduced at relapse but not significantly affected during overt disease.

To further characterize the lung inflammatory infiltrates, we examined lung histology in H&E sections (Fig. 2). Secondary aerosol challenge induced a relapse characterized by intense infiltrates containing many lymphocytes, macrophages, few plasma cells, few multinucleated giant cells, and approximately 30% eosinophils (Fig. 2A). Mice treated with DEX had reduced inflammation, particularly reduced eosinophil infiltration at relapse (Fig. 2B). However, some less dense infiltrates containing eosinophils, lymphocytes, and macrophages persisted despite treatment (Fig. 2B, insert). Overt disease was characterized by approximately 30% eosinophils within the infiltrates with high numbers of lymphocytes, macrophages, and many plasma cells compared to relapse (Fig. 2C). Unlike the effect of DEX upon relapsing airway inflammation, DEX almost eliminated overt inflammatory infiltrates, in particular eliminated eosi-

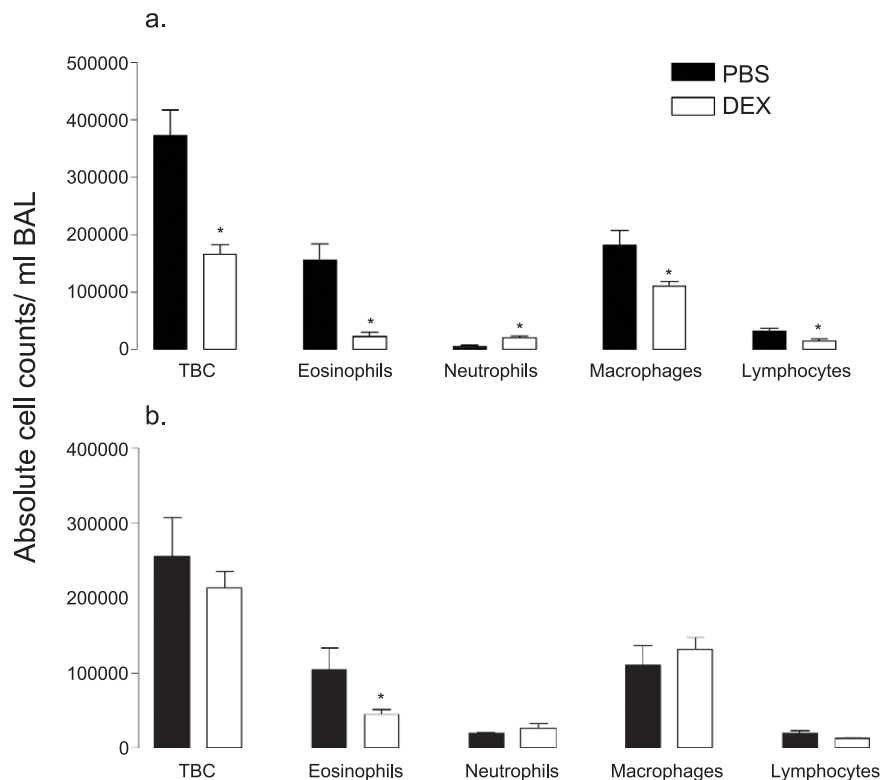


Fig. 1. Leukocyte composition in BAL samples from mice treated with inhaled DEX (10 mg%) or PBS (a) prior to relapse and (b) during overt phases of disease. TBC and absolute counts/ml of BAL from mice at relapse on day 48 and during overt disease on day 39. Values are expressed as mean $\pm$ SEM. Data presented are from five independent experiments ($n = 12-16$). Unpaired Student's *t* test ($*P < 0.05$) was used to compare differences between each group and PBS-treated controls.

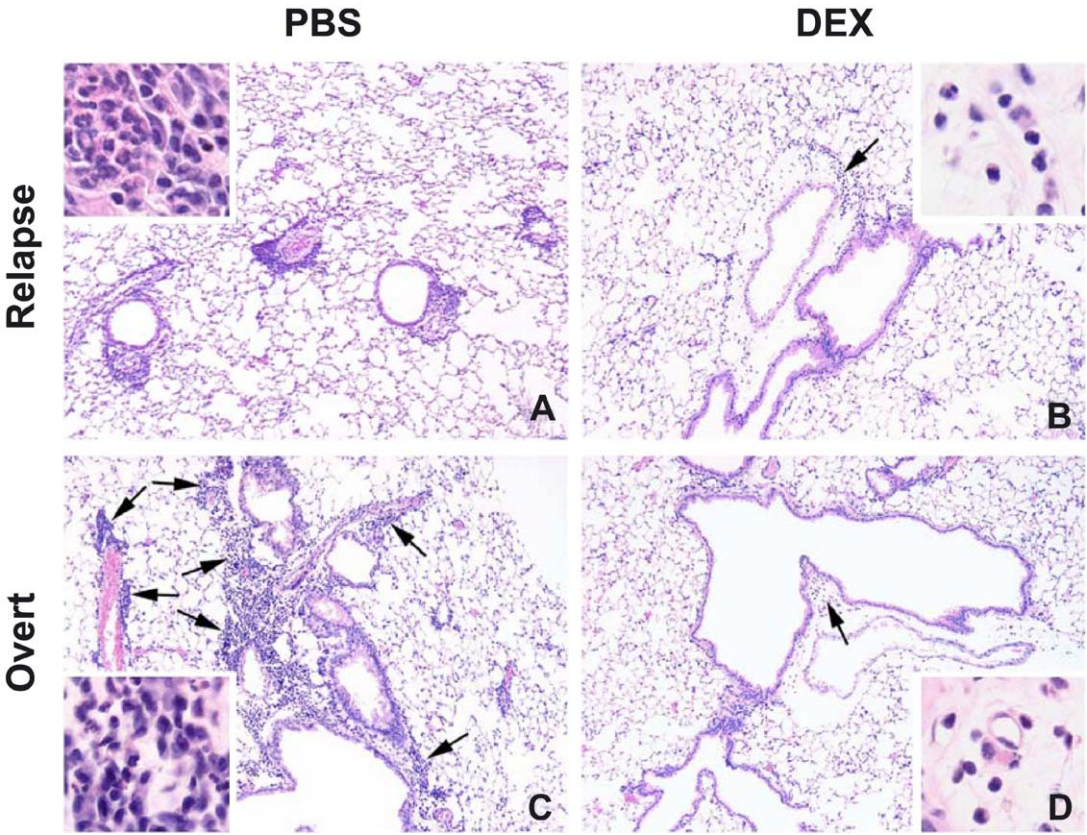


Fig. 2.

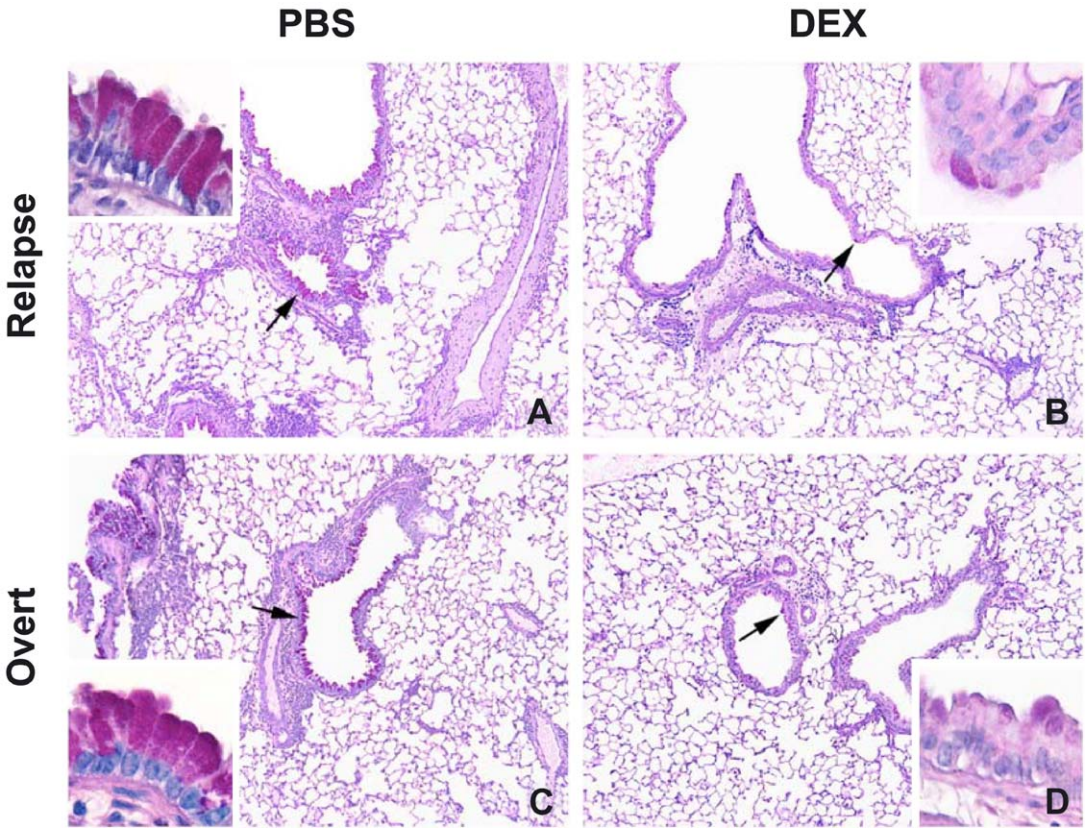


Fig. 4.

nophils (Fig. 2D). Only one area, shown in Fig. 2D, contained a few scattered cells. Quantitative evaluation of lung sections revealed that DEX treatment reduced inflammation by 75% and by less than 1% in overt compared to relapse (Fig. 3). These data demonstrate that DEX effectively reduces inflammation within the lungs during overt disease but is less efficient during relapse.

Effect of DEX treatment on mucus production

To determine whether mucus hypersecretion was modulated by steroid treatment, adjacent lung sections were stained with PAS. Lungs from naïve mice generally reveal the rare PAS-positive cell in the respiratory epithelium compared to 100% of the central airway epithelium producing mucus during relapse and overt disease [4]. Disease relapse was characterized by high levels of mucus-producing cells, goblet cell hyperplasia, and hypertrophy (Fig. 4A). During overt disease, these features, in addition to mucus plugs in the airway lumen, were more evident (Fig. 4B). DEX treatment prevented mucus production to near baseline levels when administered before relapse (Fig. 4C). In contrast, we observed that nebulized DEX during overt disease reduced the amount of mucus per cell, though many epithelial cells remained PAS-positive (Fig. 4D). Additionally, mucus plugs within the airway lumen were absent (Fig. 4D). These results indicate that inhaled steroids effectively attenuate mucus production in established allergic asthma.

Effect of DEX treatment on methacholine-induced airway hyperresponsiveness

To determine whether DEX inhibits AHR, we measured methacholine-induced airway resistance using total body plethysmography. PBS treated mice during relapse and overt disease were significantly higher ($P < 0.05$) than alum alone controls (Fig. 5). DEX treatment abolished the development of airway hyperreactivity for both relapse ($P < 0.05$) and overt disease ($P < 0.05$) compared to PBS treated controls by a factor of 1.5 (Fig. 5). These findings illustrate that inhaled steroid treatment inhibits AHR.

Effect of DEX treatment on serum and BAL immunoglobulins

Elevated levels of Th2-associated Ig are present in the serum and BAL of mice with overt and relapse disease [4].

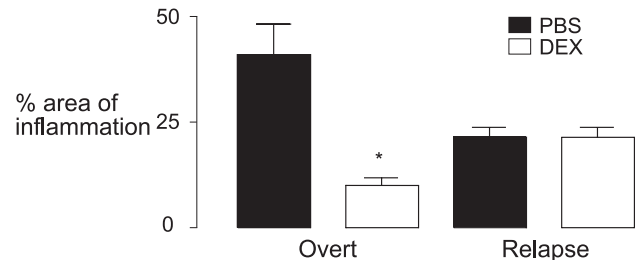


Fig. 3. Intensity of inflammatory infiltrates in lungs of mice treated with nebulized DEX during relapse and overt disease. Area of inflammation was evaluated on H&E lung sections from PBS- and DEX-treated mice during relapse and overt disease. Data are expressed as mean percentages $\pm$ SEM of inflammatory cell infiltrates within three most dense infiltrates within an ocular grid consisting of 100 squares ($5929 \mu\text{m}^2/\text{square}$) per section, excluding vascular and airway lumina from two independent experiments ($n = 12$). * $P < 0.05$ for DEX-treated compared with PBS-treated mice during overt disease.

To determine whether OVA-specific IgG1 and IgE can be inhibited by inhaled DEX treatment, we tested sera following the last aerosol. Serum IgG1 levels were unaffected by DEX treatment for either disease phase (Fig. 6). However, we observed that serum IgE was reduced by 40% at relapse but was unaffected when treatment was administered during overt disease. Inhaled DEX effectively diminished IgG1 and IgE in BAL for both disease phases. Inhibition was 75% and 77% for BAL IgE and 30% and 35% for BAL IgG1 concentrations in overt and relapse disease, respectively. To determine whether higher doses of DEX inhibited Ig, we increased the dose to 30 mg%. We observed that inhibition of BAL IgG1 production was twice that with DEX 10 mg% and that serum IgG1 was inhibited during overt disease (data not shown). These results demonstrate that DEX more effectively inhibits BAL compared to serum Ig, IgE compared to IgG1, and relapse compared to overt disease.

Discussion

Patients with allergic asthma develop chronic or intermittent disease resulting from either persistent or periodic allergen exposure, respectively. We generated allergic asthma in mice, which reflects the relapsing, remitting, and constant nature of this syndrome. Mice rechallenged with aerosol OVA during the remission or administered repeated aerosol challenges developed relapse and overt disease, respectively. Disease was characterized by AHR, progres-

Fig. 2. Lung histology from mice treated with nebulized DEX during relapse and overt disease. Representative photomicrographs of H&E whole lung sections from (A) PBS-treated relapse (day 48), (B) nebulized DEX 10 mg% treated relapse (day 48), (C) PBS-treated overt disease, (D) Nebulized DEX 10% treated overt disease (day 29). Objective lens $\times 100$ for low power field and $\times 400$ for high power field inserts. Arrows indicate areas of inflammation. High power field insert in B demonstrates the persistence of eosinophils.

Fig. 4. Mucus production from mice treated with nebulized DEX during relapse and overt disease. Representative photomicrographs of PAS-stained whole lung sections from mice with (A) PBS-treated relapse (day 48), (B) nebulized DEX 10 mg% treated relapse (day 48), (C) PBS-treated overt disease, (D) Nebulized DEX 10 mg% treated overt disease (day 29). Objective lens $\times 400$. Arrows indicate intense pink staining illustrating mucus within the respiratory epithelial goblet cells.

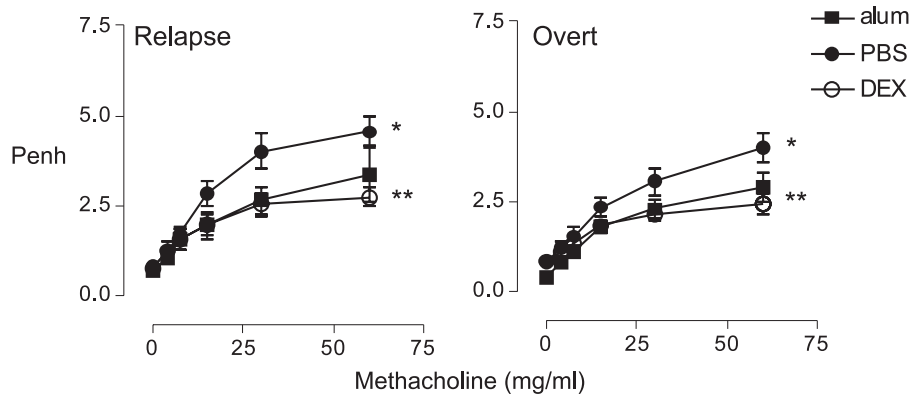


Fig. 5. Effect of nebulized DEX treatment on methacholine-induced airway hyperresponsiveness in mice during relapse and overt disease. Titrated doses of aerosolized methacholine-induced AHR was measured with a whole body plethysmography equipment 24 h after aerosol challenge for relapse (day 43) and overt (day 28) for PBS- and DEX-treated mice. Open circles represent DEX-treated mice, closed circles represent PBS-treated mice, and squares represent alum-immunized negative controls. Data are expressed as mean Penh $\pm$ SEM and from two independent experiments ($n = 12-16$). * $P < 0.05$ for PBS-treated relapse and overt mice compared with alum mice. ** $P < 0.05$ for DEX-treated relapse and overt mice compared with PBS-treated mice.

sive increases in BAL Ig, mucus production, and a transition from acute to chronic inflammation. We chose inhaled glucocorticoids as a “gold standard” and observed effective prevention of relapse AHR, mucus hypersecretion, Ig, and reduced airway inflammation. In contrast, DEX treatment during overt disease resulted in reductions in BAL Ig, mucus hypersecretion, moderate reduction in airway inflammation but complete abrogation of AHR and lung parenchyma inflammation. Thus, we illustrate that these models can be effectively treated with glucocorticoids and that distinct phases of disease respond differently to treatment.

Mice rechallenged with aerosol OVA during remission developed mucus hypersecretion, AHR, increased Ig titers, BAL inflammation, and eosinophilia, which peaked 6 days after aerosol reexposure [4]. Thus, we chose to examine this time point for DEX treatment effects. DEX effectiveness on day 44 mirrored the results we observed on day 48 during relapse but the magnitude of the response was lower (data not shown). The reason for a latter peak response in relapse compared with overt disease is unclear. We found that overall BAL inflammation was significantly prevented in DEX treatment groups during relapse, accounted for by a reduction in eosinophils, lymphocytes, and macrophages, which is also seen in human disease [10,11,21–26]. We observed that DEX consistently increased BAL neutrophils in absolute numbers, though similar to humans, BAL neutrophils are not prominent in allergic asthma in mice. However, the increased neutrophil infiltration was significant and is probably related to the effect of glucocorticoids, which are known to increase peripheral neutrophil counts due to increased margination and possibly due to inhibition of neutrophil apoptosis leading to increased survival [27,28]. DEX treatment during overt disease leads to disparate results. We observed that DEX significantly reduced eosinophil infiltration in the airway but did not affect other leukocyte populations. One possible explanation for these

contrasting results may be that prevention of airway leukocyte infiltration is easier during remission compared to active, ongoing infiltration.

In contrast to airway inflammation, inflammatory infiltrates seen on lung sections were almost eliminated with DEX treatment during overt disease but were only minimally reduced at relapse. We hypothesize that highly activated cells within the lung parenchyma during overt disease may be more sensitive to the effects of glucocorticoids than those at rest. This might potentially result in increased apoptosis. However, this would not explain the lack of DEX effect on BAL cells. An additional possibility is a DEX-induced increase in cell trafficking. DEX is known to effect adhesion molecule, chemokine, and chemokine receptor expression [9,29–31], thus increasing emigration from lung parenchyma and potentially increasing cell numbers in the airways. Another possible explanation is that plasma cells, which comprise a large percentage of the overt infiltrates and are sensitive to the effects of glucocorticoids, may be involved in maintaining the integrity of the infiltrates. Thus, when they are eliminated by DEX, the remaining infiltrating cells may be released and migrate into the airways. Thus, augmented cell migration from the interstitium may explain why there is an absence of parenchymal infiltrates and a concomitant increase of cells within the airways. Our results demonstrate that there is greater effect of DEX treatment on lung inflammation during overt disease compared with relapse that would not be recognized if lung inflammation was evaluated on BAL rather than on tissue sections.

Several studies have shown that chronic inflammation leads to airway remodeling and irreversible lung damage [7,32–36]. It is mostly likely that perivascular and peribronchial infiltrates within the epithelium and submucosa are responsible for tissue damage. Furthermore, it is not clear what role BAL cells perform in terms of pathogenesis and ultimate lung damage. Thus, cells within lung paren-

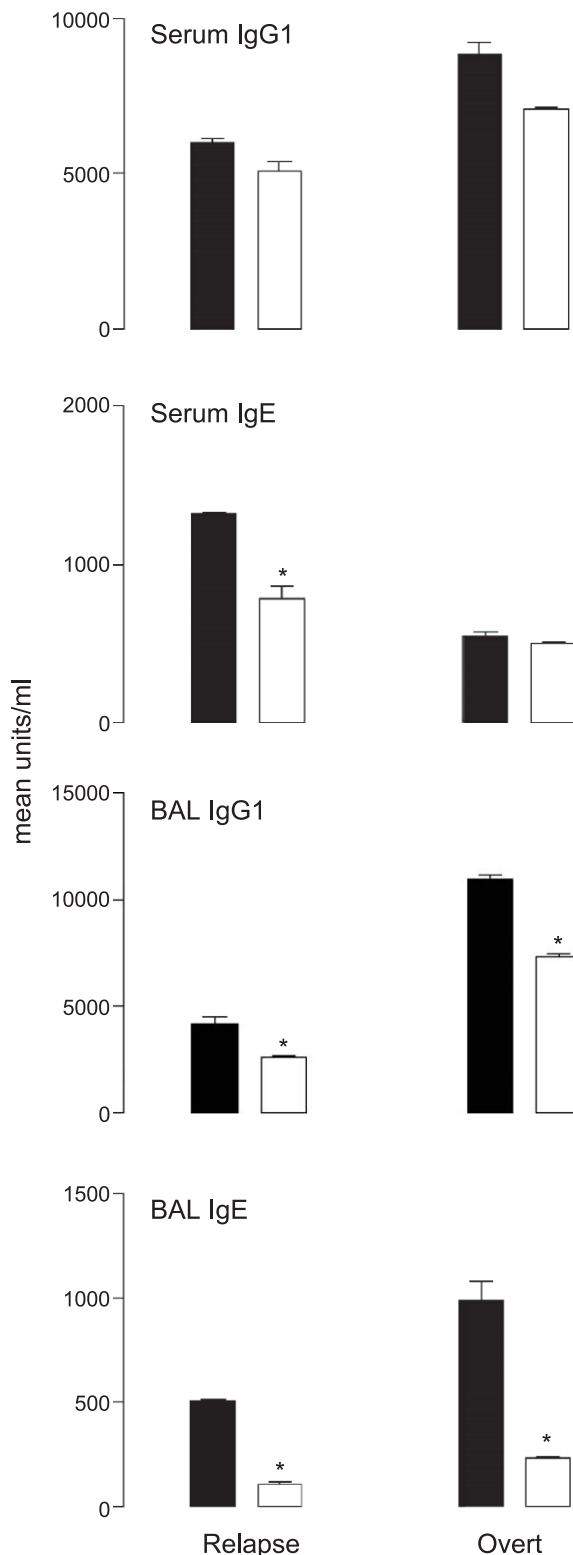


Fig. 6. Effect of nebulized DEX on Ig from mice during relapse and overt disease. Serum and BAL OVA-specific IgG1 and IgE were measured on day 48 for relapse and day 29 during overt disease. Filled and open columns represent PBS-treated mice and DEX-treated mice, respectively. Data are presented as averages of Eu^{3+} release $\pm$ SEM. Results are from two independent experiments ($n = 6$). All samples were tested in the same assay. * $P < 0.05$ was considered significant for DEX-treated relapse and overt mice compared with PBS-treated mice.

chymal infiltrates are possibly directly involved with disease pathogenesis and lung tissue destruction compared to airway cells. This suggests that it may be more useful to evaluate treatment efficacy based on lung infiltrates in animal models than BAL infiltrates.

Inhaled steroids modulated the production of OVA-specific IgG1 and IgE. BAL Ig production was particularly sensitive to the effects of DEX. In experiments using intraperitoneal administration of DEX (1 mg/kg, twice daily on 5 consecutive days), Ig was not reduced (data not shown). This suggests that inhaled DEX may directly inhibit the large number of lung plasma cells in both relapse and overt disease and may be a result of a high local concentration of DEX during inhalation treatment. There was also a reduction in serum Ig in relapse, which suggests that inhaled DEX prevents differentiation of B lymphocytes either in lungs or lymphoid organs, though it is not yet obvious whether lung plasma cells contribute substantially to serum Ig levels. We observed that IgG1 levels were a magnitude higher than IgE and that IgE was more profoundly reduced compared to IgG1. We speculate that this may correlate with higher numbers of IgG1- compared with IgE-producing plasma cells and additionally, may relate to the half-life differences between the isotypes. Moreover, the absence of a DEX effect on overt serum Ig may be related to the many plasma cells in the lungs, especially if these cells contribute to the serum Ig levels or if there is also an increase in plasma cells in other lymphoid organs. Taken together, these results suggest that the magnitude of the DEX effect on antibody production may correlate with the number of lung plasma cells and may augment the glucocorticoid effect on disease inhibition.

DEX inhibited goblet cell hyperplasia and mucus hypersecretion and AHR, which are a consequence of the OVA-specific immune response leading to inflammation and antibody production. While the DEX treatment effect on these disease parameters may be correlated with reduction in inflammation and Ig, steroids may directly inhibit mucus production by inhibiting submucosal gland cells or the expression of mucin genes [12,37,38] and AHR by reducing responsiveness to histamine, cholinergic agonists, allergen, and other spasmogens [13,14]. DEX treatment completely prevented mucus hypersecretion during relapse induction. However, PAS-stained sections of overt lungs revealed high levels of mucus, though much lower than in PBS-treated mice. Although we observed mucus in the goblet cells after treatment with DEX, this may reflect the kinetics of goblet cell mucus secretion, cell turnover, and the time required to resolve goblet cell hyperplasia rather than ongoing goblet cell hypersecretion. These results demonstrate that either by direct or indirect mechanisms of action, glucocorticoids inhibit mucus production and AHR in relapse and established overt disease.

In conclusion, we have shown that established experimental allergic asthma in mice can be attenuated with corticosteroids. DEX inhibits inflammation, mucus hyper-

secretion, and Ig differentially in relapse and overt disease phases and abolishes AHR effectively in both. These models illustrate the importance to study the effects of known reference drugs in animal models, especially in distinct phases of disease that resemble the clinical course observed in humans.

Acknowledgments

We thank Michaela Benkovszky for technical assistance, Dr. Ekke Liehl for critical reading of the manuscript, and Peter Mayer and Louise Pickart from the Novartis Forschungsinstitut GmbH, Vienna, Austria, for technical contributions. This work was funded in part by the Novartis Forschungsinstitut GmbH, Vienna, Austria, and grants from the Foundation of the Federal Reserve National Bank of Austria (8675), Foundation for Medical Sciences from the Mayor of the City of Vienna, and the Austrian Science Fund (P-14198MED). Paiboon Jungsuwadee is a scholar of the Austrian Exchange Service.

References

- [1] W. Busse, J.B. Sedgwick, Eosinophils in asthma, *Ann. Allergy* 68 (1992) 286–290.
- [2] C. Corrigan, A.B. Kay, The roles of inflammatory cells in the pathogenesis of asthma and of chronic obstructive pulmonary disease, *Am. Rev. Respir. Dis.* 143 (1991) 1165–1168.
- [3] M. Sears, The definition and diagnosis of asthma, *Allergy* 48 (1993) 12–16.
- [4] P. Jungsuwadee, G. Dekan, G. Stingl, M.M. Epstein, Recurrent aerosol antigen exposure induces distinct patterns of experimental allergic asthma in mice, *Clin. Immunol.* 102 (2002) 145–153.
- [5] B. Cronstein, G. Weissmann, Targets for antiinflammatory drugs, *Annu. Rev. Pharmacol. Toxicol.* 35 (1995) 449–462.
- [6] P.J. Barnes, Inhaled glucocorticoids for asthma, *N. Engl. J. Med.* 332 (1995) 868–875.
- [7] J. de Kluijver, C.E. Evertse, J.A. Schrumpf, H. van der Veen, A.H. Zwiderman, P.S. Hiemstra, K.F. Rabe, P.J. Sterk, Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids, *Am. J. Respir. Crit. Care Med.* 166 (2002) 294–300.
- [8] A. Ray, K.E. Prefontaine, Physical association and functional antagonism between the p65 subunit of transcription factor NF- κ B and the glucocorticoid receptor, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 752–756.
- [9] R. Schleimer, Effects of glucocorticosteroids on inflammatory cells relevant to their therapeutic applications in asthma, *Am. Rev. Respir. Dis.* 141 (1990) S59–S69.
- [10] L. Laitinen, A. Laitinen, T.A. Haahtela, A comparative study of the effects of an inhaled corticosteroid, budesonide, and of a 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial, *J. Allergy Clin. Immunol.* 90 (1992) 32–42.
- [11] R. Djukanovic, J.W. Wilson, K.M. Britten, et al., Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma, *Am. Rev. Respir. Dis.* 145 (1992) 669–674.
- [12] S. Shimura, T. Sasaki, K. Ikeda, K. Yamauchi, H. Sasaki, T. Takishima, Direct inhibitory action of glucocorticoid on glycoconjugate secretion from airway submucosal glands, *Am. Rev. Respir. Dis.* 141 (1990) 1044–1049.
- [13] P. Barnes, Effect of corticosteroids on airway hyperresponsiveness, *Am. Rev. Respir. Dis.* 141 (1990) S70–S76.
- [14] E. Bel, M.C. Timmers, A.H. Zwiderman, J.H. Dijkman, P.J. Sterk, The effect of inhaled corticosteroids on the maximal degree of airway narrowing to methacholine in asthmatic subjects, *Am. Rev. Respir. Dis.* 143 (1991) 109–113.
- [15] W.L.J. Elwood, P.J. Barnes, K.F. Chung, Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown–Norway rats, *Am. Rev. Respir. Dis.* 145 (1992) 1289–1294.
- [16] J.J. De Bie, E.M. Hessel, I. Van Ark, B. Van Esch, G. Hofman, F.P. Nijkamp, A.J. Van Oosterhout, Effect of dexamethasone and endogenous corticosterone on airway hyperresponsiveness and eosinophilia in the mouse, *Br. J. Pharmacol.* 119 (1996) 1484–1490.
- [17] M. Mathur, K. Herrmann, X.T. Li, Y.M. Qin, J. Weinstock, D. Elliott, J. Monahan, P. Padrid, TRFK-5 reverses established airway eosinophilia but not established hyperresponsiveness in a murine model of chronic asthma, *Am. J. Respir. Crit. Care Med.* 159 (1999 Feb.) 580–587.
- [18] D.I. Blyth, T.F. Wharton, M.S. Pedrick, T.J. Savage, S. Sanjar, Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-Interleukin-5 antibody, *Am. J. Respir. Cell Mol. Biol.* 23 (2000) 241–246.
- [19] N.J. Vanacker, E. Palmans, J.C. Kips, R.A. Pauwels, Fluticasone inhibits but does not reverse allergen-induced structural airway changes, *Am. J. Respir. Crit. Care Med.* 163 (2001) 674–679.
- [20] A. Trifilieff, A. El-Hashim, C. Bertrand, Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment, *Am. J. Physiol.* 279 (2000) L1120–L1128.
- [21] P. Jeffery, R.W. Godfrey, E. Ädelroth, F. Nelson, A. Rogers, S.-Å. Johansson, Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma: a quantitative light and electron microscopic study, *Am. Rev. Respir. Dis.* 145 (1992) 890–899.
- [22] C. Burke, C.K. Power, A. Norris, A. Condez, B. Schmekel, L.W. Poulter, Lung function and immunopathological changes after inhaled corticosteroid therapy in asthma, *Eur. Respir. J.* 5 (1992) 73–79.
- [23] C. Trigg, N. Manolitsas, J. Wang, M. Calderon, A. McAulay, S. Jordan, M. Herdman, N. Jhalli, J. Duddle, S. Hamilton, Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma, *Am. J. Respir. Crit. Care Med.* 150 (1994) 17–22.
- [24] M. Duddridge, C. Ward, D.J. Hendrick, E.H. Walters, Changes in bronchoalveolar lavage inflammatory cells in asthmatic patients treated with high dose inhaled beclomethasone dipropionate, *Eur. Respir. J.* 6 (1993) 489–497.
- [25] E. Ädelroth, L. Rosenhall, S.-A. Johansson, M. Linden, P. Venge, Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage, *Am. Rev. Respir. Dis.* 142 (1990) 91–99.
- [26] J.W. Wilson, R. Djukanovic, P.H. Howarth, S.T. Holgate, Inhaled beclomethasone dipropionate downregulates airway lymphocyte activation in atopic asthma, *Am. J. Respir. Crit. Care Med.* 149 (1994) 86–90.
- [27] G. Cox, Glucocorticoid treatment inhibits apoptosis in human neutrophils, *J. Immunol.* 193 (1995) 4719–4725.
- [28] L.C. Meagher, J.M. Cousin, J.R. Seckl, C. Haslett, Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes, *J. Immunol.* 156 (1996) 4422–4428.
- [29] B.N. Cronstein, S.C. Kimmel, R.I. Levin, F. Martiniuk, G. Weissmann, A mechanism for the anti-inflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial–leukocyte adhesion molecule 1 and intercellular adhesion molecule 1, *Proc. Natl. Acad. Sci. U. S. A.* 89 (1992) 9991–9995.
- [30] A. van de Stolpe, E. Caldenhoven, J.A. Raaijmakers, P.T. van der Saag, L. Koenderman, Glucocorticoid-mediated repression of intercellular adhesion molecule-1 expression in human monocytic and bronchial epithelial cell lines, *Am. J. Respir. Cell Mol. Biol.* 8 (1993) 340–347.

- [31] F.L. Jahnsen, R. Haye, E. Gran, P. Brandtzaeg, F.-E. Johansen, Glucocorticosteroids inhibit mRNA expression for eotaxin, eotaxin-2, and monocyte–chemotactic protein-4 in human airway inflammation with eosinophilia, *J. Immunol.* 163 (1999) 1545–1551.
- [32] C.M. Hogaboam, K. Blease, B. Mehrad, M.L. Steinhauser, T.J. Standiford, S.L. Kunkel, N.W. Lukacs, Chronic airway hyperreactivity, goblet cell hyperplasia, and peribronchial fibrosis during allergic airway disease induced by *Aspergillus fumigatus*, *Am. J. Pathol.* 156 (2000) 723–732.
- [33] J. Temelkovski, S.P. Hogan, D.P. Shepherd, P.S. Foster, R.K. Kumar, An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen, *Thorax* 53 (1998) 849–856.
- [34] C.A. Yiamouyiannis, C.M. Schramm, L. Puddington, P. Stengel, E. Baradaran-Hosseini, W.W. Wolyniec, H.E. Whiteley, R.S. Thrall, Shifts in lung lymphocyte profiles correlate with the sequential development of acute allergic and chronic tolerant stages in a murine asthma model, *Am. J. Pathol.* 154 (1999) 1911–1921.
- [35] R.K. Kumar, P.S. Foster, Modeling allergic asthma in mice: pitfalls and opportunities, *Am. J. Respir. Cell Mol. Biol.* 27 (2002) 267–272.
- [36] E. Palmans, J.C. Kips, R.A. Pauwels, Prolonged allergen exposure induces structural airway changes in sensitized rats, *Am. J. Respir. Crit. Care Med.* 161 (2000) 627–635.
- [37] J.D. Lundgren, F. Hirata, Z. Marom, C. Logun, L. Steel, M. Kaliner, J. Shelhamer, Dexamethasone inhibits respiratory glycoconjugate secretion from feline airways in vitro by the induction of lipocortin (lipomodulin) synthesis, *Am. Rev. Respir. Dis.* 137 (1988) 353–357.
- [38] H. Kai, K. Yoshitake, A. Hisatsune, T. Kido, Y. Isohama, K. Miyata, T. Miyata, Dexamethasone suppresses mucus production and MUC-2 and MUC-5AC gene expression by NCI-H292 cells, *Am. J. Physiol.* 271 (1996) L484–L488.