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Prevention and reversal of pulmonary inflammation and airway hyperresponsiveness by dexamethasone treatment in a murine model of asthma induced by house dust

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Kim, Jiyoun, Laura McKinley, Javed Siddiqui, Gerry L. Bolgos, and Daniel G. Remick. Prevention and reversal of pulmonary inflammation and airway hyperresponsiveness by dexamethasone treatment in a murine model of asthma induced by house dust. *Am J Physiol Lung Cell Mol Physiol* 287: L503–L509, 2004. First published May 7, 2004; 10.1152/ajplung.00433.2003.—The morbidity and mortality from asthma in the Western world have increased 75% in the past 20 years. Recent studies have demonstrated that sensitization to cockroach allergens correlates strongly with the increased asthma morbidity for adults and children. We investigated whether dexamethasone administered before or after allergen challenge would inhibit the pulmonary inflammation and airway hyperresponsiveness in a mouse model of asthma induced by a house dust extract with high levels of cockroach allergens. For the prevention experiment, mice were treated with an intraperitoneal injection of dexamethasone 1 h before each pulmonary challenge, and airway hyperresponsiveness was measured 24 h after the last challenge. Mice were killed 48 h after the last challenge. For the reversal study, airway hyperresponsiveness was measured 24 h after the last challenge, and the mice were treated with dexamethasone. Dexamethasone treatment before allergen challenge significantly reduced the pulmonary recruitment of inflammatory cells, myeloperoxidase activity in the lung, airway hyperreactivity, and total serum IgE levels compared with PBS-treated mice. Additionally, dexamethasone treatment could significantly reduce the airway hyperreactivity of an established asthmatic response. These results demonstrate that dexamethasone not only prevents but also halts the asthmatic response induced by house dust containing cockroach allergens. This model exhibits several features of human asthma that may be exploited in the study of pathophysiological mechanisms and potential therapeutic interventions.

corticosteroids; eosinophils; neutrophils; airway hyperreactivity; IgE

ASTHMA IS A UNIQUE FORM of chronic airway inflammation characterized by reversible airway obstruction, inflammatory mediator production, and airway hyperresponsiveness (AHR) (34). After exposure to allergens, the airway is infiltrated with a variety of inflammatory cells, including lymphocytes, macrophages, neutrophils, and eosinophils. Among these, eosinophils are the predominant effector cells for tissue damage and pulmonary dysfunction (34, 41). Furthermore, the intensity of pulmonary recruitment of eosinophils correlates strongly with the severity of AHR (18, 21, 48).

Once eosinophils have infiltrated the lung, numerous inflammatory changes in the airways are triggered, including the release of a wide variety of immunomodulator molecules such as major basic protein (35, 41). The localization of eosinophils to the bronchial mucosa potentially primes the lung for subsequent immune responses and augments allergic pulmonary inflammation by the secretion of various cytokines (8, 34). Selective recruitment of eosinophils into the airways during allergic inflammation suggests that eosinophil-specific chemoattractants are produced and released throughout the course of pulmonary inflammation. The C-C chemokine eotaxin is considered the major eosinophil chemoattractant in animal models of eosinophilic pulmonary inflammation (17, 36) and in human tissues (15, 31) after allergen sensitization.

An increase in AHR in response to a methacholine challenge has been demonstrated as a diagnostic sign of asthma in various animal models of asthma (48). Enhanced pause (Penh) from whole body plethysmography in unrestrained and conscious animals represents a widely used measure of AHR, and such changes are strongly correlated with pulmonary recruitment of inflammatory cells in asthmatic animals (19).

Glucocorticoids are currently the most effective treatment for asthma with proven effectiveness and safety (40, 43), and the efficacy of these agents has been demonstrated in the prevention of asthma morbidity and mortality (43). Routine use of glucocorticoids as a prophylactic measure of asthma has improved disease outcomes, including reduced hospitalizations (47). Furthermore, early treatment of acute asthma with systemic administration of corticosteroids for emergency department patients has dramatically reduced the need for hospitalization, prevented relapse, and expedited recovery, especially for patients with severe asthma and for children (37, 38).

Various mouse models of asthma have been developed for studying the inflammatory mechanisms of asthma (5). To induce allergic asthma-like pulmonary inflammation in healthy animals, it was necessary to sensitize and challenge with specific allergens. Among them, ovalbumin (13, 20) and purified indoor allergens such as cockroach (9, 49) and dust mite (11, 44) are commonly used allergens in murine asthma models. However, in terms of quality and quantity of allergens, the allergens used for these animal models may not represent exactly the same constituents to which asthmatics are exposed throughout their daily lives. To date, very few environmental allergens collected directly from houses have been used to develop animal models of asthma-like pulmonary inflammation (24). We have developed a novel murine model of allergic pulmonary inflammation (24) that shows AHR, bronchopulmonary recruitment of inflammatory cells, and pulmonary expression of chemokines following house dust extract immunization and challenge.

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This model may be exploited further to examine therapeutic modalities to treat asthma. As a first step in this investigation, we sought to determine whether a classic treatment option for acute asthma, glucocorticoids, would prevent or break an asthmatic response in this model. The animals were treated with the glucocorticosteroid dexamethasone before or after the onset of an asthmatic response to determine the effects of corticosteroids in the pulmonary infiltration of inflammatory cells and bronchopulmonary hyperresponsiveness.

MATERIALS AND METHODS

Mice

Female BALB/c mice (18–20 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and maintained under standard laboratory conditions. The mice were housed in a temperature-controlled room (22°C) with a 12:12-h light-dark cycle with food and water allowed ad libitum. All experiments were performed in accordance with the National Institutes of Health guidelines and approved by the University of Michigan Animal Use Committee.

Experiment Design

The household dust used for all sensitizations and airway challenges was collected from a house in Detroit, MI, and then extracted as we previously reported (24). Briefly, a total of 4.3 g of house dust was collected from the house and extracted with 30 ml of sterile PBS. This house dust extract was assayed for nine different allergens including six indoor and three outdoor allergens: German cockroach (*Blattella germanica*, Bla g1 and Bla g2), house dust mite (*Dermatophagoides pteronyssinus* Der p1, and *Dermatophagoides farinae* Der f1), cat (*Felis domesticus*, Fel d1), and dog (*Canis familiaris*, Can f1), meadow fescue (*Festuca pratensis*), short ragweed (*Ambrosia artemisiifolia*), and mold (*Alternaria alternata*). Our house dust extract contained very high concentrations of cockroach allergens (378 U/ml Bla g1 and 6,249 ng/ml Bla g2), whereas four other indoor allergens and all three outdoor allergens were very low (data not shown). The house dust extract contained 270 pg/ml of endotoxin. We used this aqueous house dust extract (diluted 1:10) for immunization and intratracheal instillation as previously described (24). Briefly, mice were sensitized by an intraperitoneal injection of 50 μl of house dust extract mixed with an adjuvant (TiterMax Gold; CytRx, Norcross, GA) for a total volume of 100 μl on *day 0* (Fig. 1). On *days 14* and *21*, mice were given a pulmonary challenge of 50 μl of house dust extract (27). For controls, normal female BALB/c mice were also examined. These mice were not immunized or challenged.

Dexamethasone Treatment

For the prevention study, immunized mice were treated with 2.5 mg/kg body wt of water-soluble dexamethasone (catalog no. D 2915; Sigma, St. Louis, MO) in PBS by intraperitoneal injection 1 h before each pulmonary challenge on *days 14* and *21* (Fig. 1). Control mice received 0.2 ml of PBS.

For the reversal study, mice were immunized and challenged twice on *days 14* and *21*. Twenty-four hours after the last challenge (*day 22*), AHR was measured, and the mice received an intraperitoneal injection of dexamethasone (2.5 mg/kg) immediately afterward (Fig. 1). Another AHR was measured 12 h and again 24 h after dexamethasone administration.

Determination of AHR

Twenty-four hours after the final challenge, AHR was measured for both the prevention and the reversal studies, as described in our previous publication (24). AHR was again measured 12 and 24 h after dexamethasone administration (36 and 48 h after the last allergen challenge, respectively) in the reversal experiment. Changes in early expiration due to bronchoconstriction were measured and expressed as Penh, which is a main indicator of airway obstruction. Airway resistance of the animal is strongly correlated with Penh and is widely accepted in murine asthma models (19). Airway responsiveness was expressed as a percent increase of Penh for each concentration of methacholine compared with Penh for PBS challenge. Increasing doses of aerosolized acetyl β-methylcholine (Sigma) were delivered for 2 min, and the response to each dose was measured for 5 min by a whole body plethysmography system (Buxco, Troy, NY) as previously reported (24).

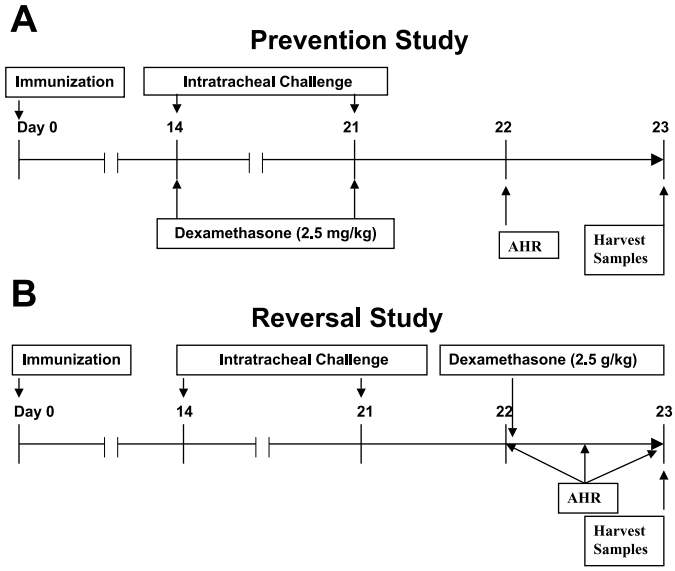

Fig. 1. Experiment protocols. Groups of female BALB/c mice received 1 immunization (*day 0*) and 2 intratracheal challenges (*days 14* and *21*) with 50 μl of house dust extract. For the prevention study, mice received dexamethasone (Dexa) treatment 1 h before each intratracheal challenge by intraperitoneal injection. Airway hyperresponsiveness (AHR) was measured 24 h after the last pulmonary challenge, and mice were killed 24 h after AHR measurement (48 h after the last challenge). For the reversal study, mice did not receive Dexa treatment with the pulmonary challenge but were treated with Dexa after the first AHR was measured. Additional measurements of AHR were preformed 12 and 24 h after the Dexa treatment. Mice were killed immediately after the last AHR was measured (48 h after the last challenge).

Sample Collection and Analysis

Forty-eight hours from the last airway challenge (*day 23*), the mice were killed for collection of blood, bronchoalveolar lavage, and histological examination as described in our previous report (24). An analysis of total IgE in mouse plasma was performed by ELISA, and the IgE standard curve was used for calculation of total IgE concentrations. We assayed the total serum IgE concentration, since a standard for cockroach allergen-specific IgE is not available.

For the myeloperoxidase assay, the right lung was removed and processed as described previously (27). Even though it is important to discriminate between eosinophils and neutrophils in the inflammatory reaction, especially in asthma (39), our myeloperoxidase assay of lung tissue homogenates detected peroxidase from neutrophils and eosinophils.

Statistical Analyses

Means ± SE were used for summary statistics in all figures. Differences between all treatment groups were compared by ANOVA. Tukey's test for pairwise comparisons was performed when the overall F value was statistically significant ($P < 0.05$).

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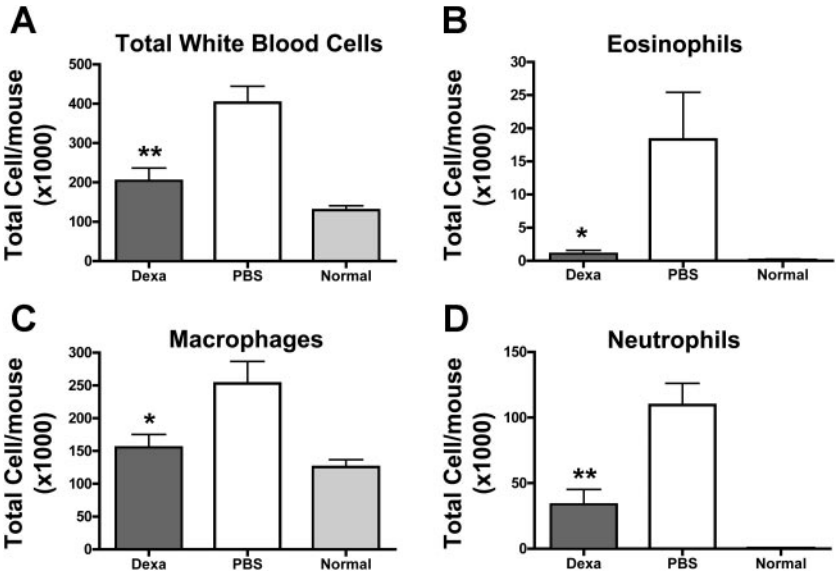

Fig. 2. Dexa decreases bronchoalveolar lavage inflammatory cell numbers. BALB/c mice received 1 immunization and 2 pulmonary challenges with the house dust extract. Mice were treated with systemic Dexa (2.5 mg/kg) or PBS 1 h before each pulmonary challenge. Normal mice did not receive any immunization, pulmonary challenge, or treatment. We performed all cell counts 48 h after the last challenge and cell differentials by counting 300 cells. Values represent means ± SE with $n = 8$ for Dexa or PBS group and $n = 3$ for normal. *$P < 0.05$ or **$P < 0.01$ compared with PBS treated.

RESULTS

Effects of Dexamethasone as a Preventative Measure

Pulmonary recruitment of inflammatory cells. In an effort to demonstrate whether corticosteroid treatment can modify the pathophysiology of asthma in a novel murine model, BALB/c mice were immunized once and challenged twice intratracheally with a house dust extract containing high concentrations of cockroach allergens (Bla g1, 37.8 U/ml; Bla g2, 625 ng/ml). Mice received a single dexamethasone treatment 1 h before each challenge and were killed 48 h after the last challenge. The house dust extract induced inflammatory cell infiltration in the bronchoalveolar lavage. The numbers of inflammatory cells in the bronchoalveolar lavage, including eosinophils, macrophages, and neutrophils in the dexamethasone-treated mice were significantly lower than those in PBS-treated mice (Fig. 2). However, dexamethasone did not decrease the number of bronchoalveolar lavage lymphocytes (data not shown). The effect of dexamethasone on pulmonary recruitment of inflammatory cells in this model was further evaluated by measurement of pulmonary myeloperoxidase activity (Fig. 3). The neutrophil and eosinophil activity within the lung tissue detected by the myeloperoxidase assay was dramatically reduced in dexamethasone-treated mice ($P = 0.001$) when compared with PBS-treated mice.

Systemic effects. We then ascertained whether the plasma IgE concentration was affected by systemic corticosteroid therapy. As seen in Fig. 4, total plasma IgE levels in dexamethasone-treated mice were substantially lower ($P < 0.001$) than levels in mice treated with PBS. In an effort to confirm that plasma expression of IgE is modified by dexamethasone treatment itself, we investigated the plasma IgE levels in three groups of mice: the mice immunized with house dust that received the dexamethasone treatment, the mice immunized with house dust that received the PBS treatment, and normal mice. There were no significant differences among three mice groups (data not shown). We also examined the changes in circulating blood cell counts, and no significant differences were observed between dexamethasone-treated mice and PBS-treated mice (data not shown).

Modification of AHR. We evaluated the effects of dexamethasone on bronchopulmonary hyperreactivity in house dust extract-immunized mice by measuring Penh via whole body plethysmography. Immunized BALB/c mice were treated with dexamethasone 1 h before each intratracheal challenge on *days 14* and *21*. AHR in response to aerosolized methacholine was measured 24 h after the last pulmonary challenge (Fig. 5). Dexamethasone treatment significantly reduced bronchopulmonary hyperresponsiveness when compared with PBS treatment ($P < 0.04$ at 25 and 50 mg/ml methacholine challenge).

Effects of Dexamethasone on Acute Asthma Attack

Glucocorticoids are frequently used for treatment of an acute asthmatic attack, i.e., the drug is given after the onset of symptoms. Therefore, we investigated whether glucocorticosteroid treatment after the onset of an asthma attack can reduce the severity of bronchopulmonary hyperresponsiveness and pulmonary recruitment of inflammatory cells in our model.

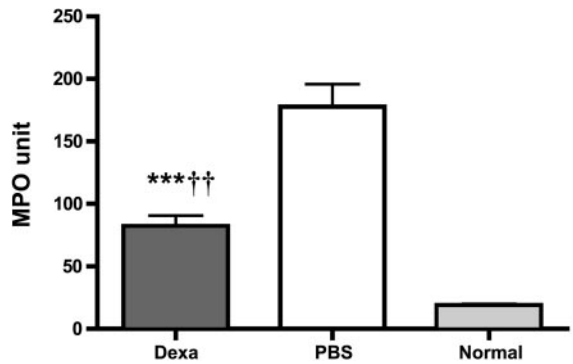

Fig. 3. Dexa decreases pulmonary myeloperoxidase (MPO) activity. BALB/c mice were immunized and challenged with the house dust extract. Mice received systemic Dexa (2.5 mg/kg) or PBS 1 h before each pulmonary challenge and were killed 48 h after the last challenge. Normal mice did not receive any immunization, pulmonary challenge, or treatment. After bronchoalveolar lavage fluid was collected, the right lung was removed and immediately processed for the assay. Values represent means ± SE with $n = 8$ for Dexa or PBS group and $n = 3$ for normal. ***$P < 0.001$ compared with PBS treated and ††$P < 0.01$ compared with normal.

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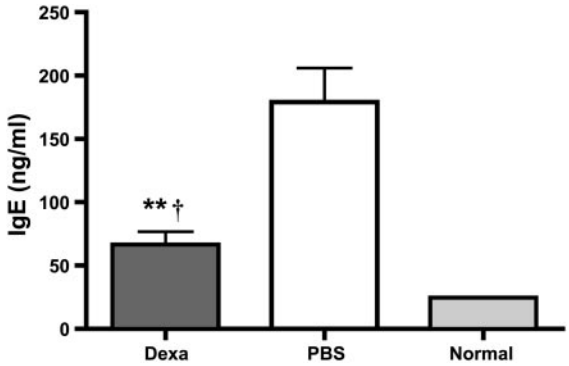

Fig. 4. Dexa decreases plasma levels of total IgE in house dust extract-sensitized and -challenged BALB/c mice. Mice received systemic Dexa (2.5 mg/kg) or PBS 1 h before each pulmonary challenge. Normal mice did not receive any immunization, pulmonary challenge, or treatment. Blood was collected 48 h after the last intratracheal challenge. Values represent means ± SE with $n = 8$ for Dexa or PBS group and $n = 3$ for normal. $^{**}P < 0.01$ compared with PBS treated and $^{\dagger}P < 0.05$ compared with normal.

Mice were immunized and then challenged twice with the house dust extract. Twenty-four hours after the last challenge, we measured AHR via whole body plethysmography. Immediately after measuring AHR, we treated one group of mice with dexamethasone while giving the control group PBS treatment. Twelve hours after the dexamethasone administration, we again measured AHR to investigate whether dexamethasone could reduce the severity of asthma attack. As shown in Fig. 6*A*, there is no significant difference between the two groups of BALB/c mice before dexamethasone treatment. However, as shown in Fig. 6*B*, dexamethasone treatment significantly reduced bronchopulmonary hyperreactivity ($P < 0.05$ at 25 and 50 mg/ml methacholine) within 12 h. This effect of dexamethasone on AHR was no longer present 24 h after administration (Fig. 6*C*). In addition, the number of neutrophils infiltrated into the airway in dexamethasone-treated group was significantly higher than in PBS-treated ($P = 0.036$), whereas the number of macrophages and eosinophils was lower when compared with PBS-treated mice (Fig. 7).

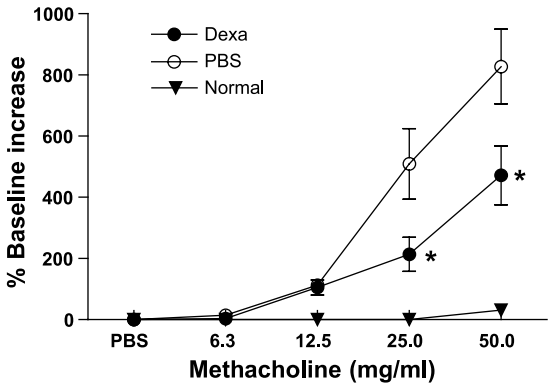

Fig. 5. AHR in Dexa- or PBS-treated mice. The Dexa (2.5 mg/kg) or PBS was administered systemically 1 h before each challenge. Normal mice did not receive any immunization, pulmonary challenge, or treatment. Enhanced pause (Penh) values were obtained from mice 24 h after the last challenge in response to nebulized methacholine via whole body plethysmography. The data are expressed as means ± SE of Penh values ($n = 8$ for Dexa or PBS and $n = 3$ for normal) as the increased percentage of baseline observed after PBS nebulization. $^{*}P < 0.05$ compared with PBS-treated group.

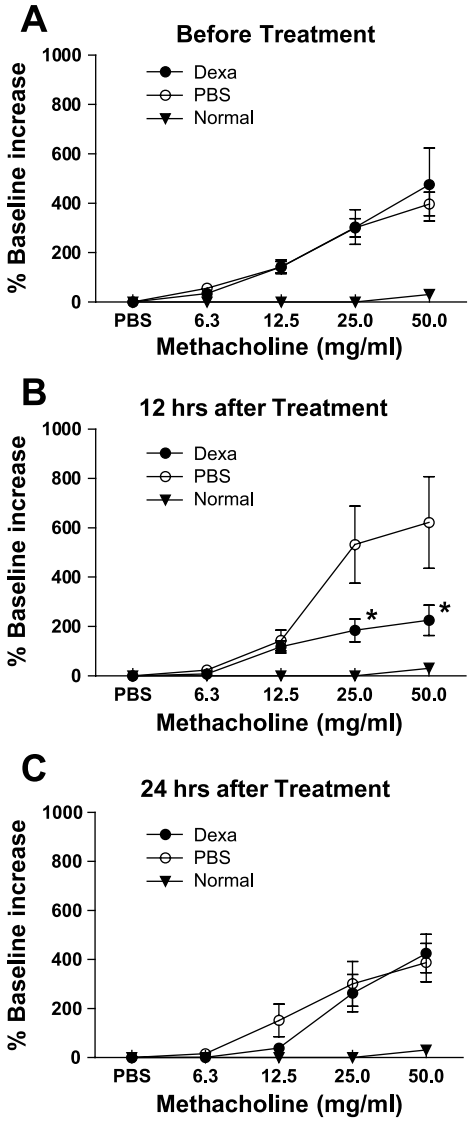

Fig. 6. Dexa breaks AHR after the onset of the asthmatic response. Groups of BALB/c mice were immunized and challenged with house dust extract. Normal mice did not receive any immunization, pulmonary challenge, or treatment. Twenty-four hours after the last challenge, AHR was measured (*A*). Immediately after the AHR, mice were treated with either systemic Dexa (2.5 mg/kg) or PBS. Twelve and twenty-four hours after the treatment, the second (*B*) and the third AHR were measured, respectively. The data are expressed as means ± SE of Penh values ($n = 8$) as the increased percentage above baseline observed after PBS nebulization. The data for the normal mice were obtained from 1 time point and used for all 3 panels. $^{*}P < 0.05$ compared with PBS-treated group.

DISCUSSION

Currently glucocorticoids, either inhaled or systemically delivered, are the most effective anti-inflammatory drugs in the treatment of asthma and are widely recommended as first-line therapy for this disease (1, 7). Regular use of corticosteroids has been shown to significantly reduce the mortality and morbidity of asthma (43).

Glucocorticosteroid suppression of inflammation occurs via inhibition of multiple aspects of the inflammatory process that include an increase in the expression of anti-inflammatory genes and proteins, as well as a decrease in the expression of

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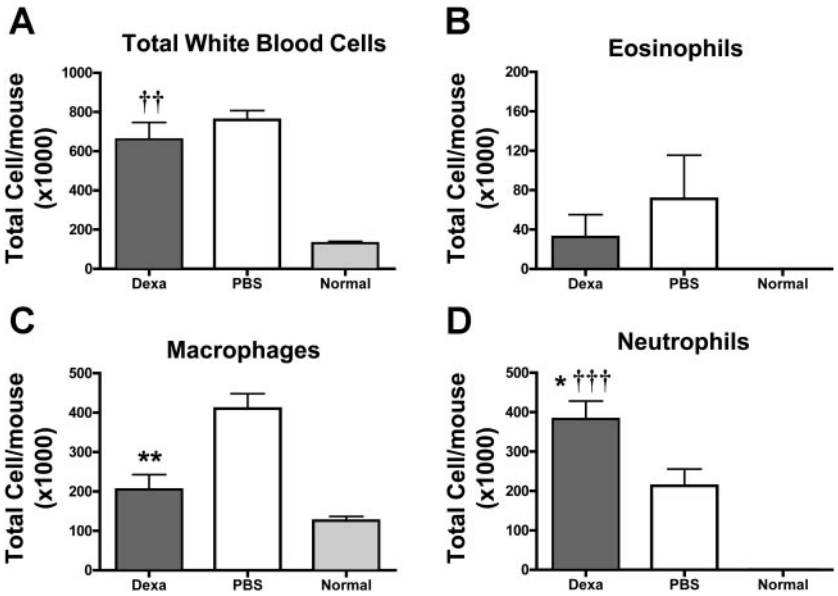

Fig. 7. Dexa alters airway infiltration of inflammatory cells after the onset of the acute asthma attack. BALB/c mice were immunized and challenged with house dust extract. Twenty-four hours after the last challenge, mice were treated with either systemic Dexa (2.5 mg/kg) or PBS. Normal mice did not receive any immunization, pulmonary challenge, or treatment. All cell counts were performed 48 h after the treatment, and white blood cell differentials were performed after 300 cells were counted. Values represent means ± SE with $n = 8$ for Dexa or PBS group and $n = 3$ for normal. $*P < 0.05$ or $**P < 0.01$ compared with PBS treated; $††P < 0.01$ or $†††P < 0.001$ compared with normal.

proinflammatory genes and proteins (1). Corticosteroids inhibit the binding of transcription factors such as nuclear factor-κB and activator protein-1 (7) to DNA. Glucocorticoids also inhibit the synthesis of inflammatory cytokines and chemokines including IL-1β, IL-2, IL-3, IL-6, IL-8, TNF-α, granulocyte-monocyte colony-stimulating factor, eotaxin, monocyte chemoattractant protein-1, and regulated on activation normal T cell expressed and presumably secreted, which are involved in chemotaxis and apoptosis of inflammatory cells such as eosinophils and lymphocytes (3, 40). Clinical studies showed that treatment of asthma with corticosteroids significantly reduced the number of eosinophils in bronchial mucosa, bronchoalveolar lavage (10, 16, 46), and circulating blood (16) by totally reversing the delayed eosinophil apoptosis in asthma (14, 23).

Pulmonary inflammation and structural changes in the airway induce AHR via the expression of inflammatory mediators including cytokines and chemokines (2, 6). These inflammatory mediators induce remodeling of asthmatic airways through the modification of the smooth muscle contractility, influx of inflammatory cells, vascular permeability, and mucus secretion (6, 22, 25). Several placebo-controlled clinical studies have demonstrated that inhaled or oral corticosteroid treatment ameliorates airway responsiveness (10, 28, 29).

We reported a novel murine model of asthma-like bronchopulmonary inflammation induced by house dust extract that contained high levels of cockroach allergens and moderate levels of lipopolysaccharide (24). This unique murine model of asthma simulates many features of human asthma, including exacerbation of AHR, pulmonary infiltration of inflammatory cells, and increased recruitment of inflammatory cells and chemokines in bronchoalveolar lavage. Although inhaled, oral, or intravenously administered glucocorticoids are the most effective treatments of asthma (1, 7), very few studies have attempted to develop an animal model to investigate the anti-inflammatory actions of this treatment.

We investigated the effects of dexamethasone, a standard glucocorticosteroid, on the features demonstrated in this model in an effort to expand the understanding of the mechanism of AHR and pulmonary inflammation. Our data clearly demonstrate that dexamethasone treatment significantly reduced various aspects of pulmonary inflammation in this model following sensitization and intratracheal challenges. Thus our mouse model closely parallels the human studies. Our results of decreased IgE concentrations in blood of dexamethasone-treated animals are consistent with other reports recently published (4, 32, 33).

Our data are similar to previous reports that demonstrated significantly smaller numbers of inflammatory cells and lower myeloperoxidase activity in the mouse model (42, 45) or in a human study (10). The fact that there was no significant difference observed between the number of inflammatory cells in blood of dexamethasone-treated mice and PBS-treated mice with significantly reduced numbers of eosinophils, macrophage, and neutrophils in the lung lavage is suggestive of a downregulation of chemotactic signals and/or reduced expression of cell adhesion molecules for these cells (30). It is noted that the number of neutrophils recruited in the lung was also reduced in the dexamethasone-treated mice, although neutrophils are generally not sensitive to the effects of glucocorticoids (1). However, studies have shown that glucocorticosteroid treatment prolonged the survival time of neutrophils secondary to decreased apoptosis (12, 26). Therefore, if dexamethasone was administered after the onset of an asthma attack, the total number of pulmonary of neutrophils was increased, while the numbers of total leukocytes, eosinophils, and macrophages were decreased. Our previously published data using the same mouse model showed an early influx of neutrophils into the bronchoalveolar lavage fluid (within 12 h after the last challenge), and the numbers peaked 36 h after the second intratracheal challenge. These data are consistent with report by Cox (12), who demonstrated that survival of neutrophils isolated from human blood was significantly increased by glucocorticoids in a dose-dependent manner. These data indicate that glucocorticoids will prevent the recruitment of neutrophils into the lung, but if they are already present, the drugs do not accelerate clearance.

In this study, we also demonstrated that systemic administration of dexamethasone significantly prevented house dust

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extract-induced bronchopulmonary hyperreactivity, which is consistent with previous clinical trials (10, 28) and animal models using different allergens (42, 45). However, the effect of dexamethasone on AHR is more dramatically demonstrated when the dexamethasone was administered after the onset of an asthma attack. There are few studies investigating the effects of glucocorticoids delivered after the onset of an asthmatic response in an animal model. Here, dexamethasone was delivered immediately after the 24-h AHR measurement, on the basis of results from the prevention study that showed an AHR peak 24 h after the last intratracheal challenge. Twelve hours after the dexamethasone treatment and 36 h after the last allergen challenge, the bronchopulmonary hyperreactivity in the treated group was significantly improved compared with untreated mice. A similar result was observed in a human study where inhaled budesonide was administered after the onset of the asthmatic response (29).

This represents a very important aspect of our model with respect to the clinical relevance to human asthma, since corticosteroids are the first choice of treatment when an asthmatic patient visits the emergency department for an acute asthma attack (37, 38). In addition, systemic administration of corticosteroids within 1 h of arrival to the emergency department greatly reduces the hospital admission time for asthmatic patients, especially those with more severe asthma, as well as patients who are not being treated with steroids (37). Early treatment also decreases the need for revisiting the clinic for additional treatment, as does the use of β-agonists (38). Our results also suggest that when we pretreat the mice with dexamethasone, the effects of dexamethasone last longer, (i.e., the Penh decrease persists for 24 h), but treatment after the onset of symptoms with dexamethasone was not as effective (i.e., the Penh decrease persists only for 12 h). This supports the current status of clinical management: that it is better to keep the asthma under control rather than only treat acute exacerbations.

Together, these results suggest that administration of dexamethasone not only prevents, but also breaks, the asthmatic pulmonary inflammation in this novel allergic murine model induced by house dust extract immunization and intratracheal instillation. We believe that the murine model of asthma described in the current study closely reproduces the characteristics of human asthma and may be an invaluable tool for further study of the immunopathophysiology of asthma and the interaction with environmental constituents.

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GRANTS

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