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.
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 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. For the prevention study, immunized and challenged 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).

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).
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.
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. 6A, there is no significant difference between the two groups of BALB/c mice before dexamethasone treatment. However, as shown in Fig. 6B, 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. 6C). 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).

**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).

Glucocorticoid 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...
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-1, 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 glucocorticoid 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
REFERENCES


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