

Inhibition of airways inflammation by dexamethasone is followed by reduced bronchial hyperreactivity in BP2 mice

S.-Y. EUM*, C. CRÉMINON†, S. HAILÉ‡, J. LEFORT* and B. B. VARGAFTIG*

*Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur, INSERM 285, †CEA, Service de Pharmacologie et d'Immunologie, DRM, Centre d'Etudes Saclay, Gif sur Yvette and ‡Unité d'Histopathologie, Institut Pasteur, Paris, France

Summary

Background Infiltration of inflammatory cells in the airways is a constant characteristic of asthma and is considered to result in bronchial hyperreactivity (BHR). We have recently developed a model of BHR using a selection of mice, named BP2, which display eosinophil-dependent BHR following antigen challenges. An anti-IL-5 antibody suppressed antigen-induced eosinophil recruitment to the airways and BHR in BP2 mice.

Objective To investigate the implication of infiltrated inflammatory cells in the induction of BHR in mice.

Methods The effects of glucocorticosteroid dexamethasone on airways eosinophilia and BHR were observed.

Results Administration of dexamethasone at the dose of 1.25 mg/kg i.p. 1 h before each of four antigen provocations suppressed the airways eosinophilia and BHR in response to intravenous 5-HT and to aerosolized methacholine, as well as IL-5 production in the BALF and in the serum. By contrast, dexamethasone failed to reduce anaphylactic bronchoconstriction.

Conclusions These results suggest that dexamethasone exerts its inhibitory effects on antigen-induced airways eosinophilia in mice by inhibiting IL-5 production, but that it does not block the liberation of anaphylactic mediators in mice.

Keywords: asthma, airways eosinophilia, corticosteroids, interleukin-5, bronchial hyperreactivity, anaphylactic bronchoconstriction, mice

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Introduction

Infiltration of inflammatory cells, particularly eosinophils, into the airways is a prominent feature of bronchial asthma [1]. Mucosal inflammation is believed to contribute to the pathogenesis of bronchial hyperreactivity (BHR), possibly because of epithelial damage caused by eosinophil-derived products [2]. Proliferation, differentiation, recruitment, survival and activation of eosinophils

are regulated by the cytokines IL-3, IL-5 and GM-CSF [3–6]. In particular, IL-5 has a selective effect on the terminal differentiation and on the recruitment of eosinophils [4].

Glucocorticosteroids, the most effective class of drugs available for the treatment of bronchial asthma, reduce eosinophil numbers in the airways of patients [7,8], and suppress *in vitro* IL-5 production by peripheral blood mononuclear cells [9]. These results suggest that glucocorticosteroids exert their clinical effect by inhibiting the production of IL-5, leading to the reduction of eosinophil production and infiltration. Recently, we developed a murine model for antigen-induced eosinophil-dependent

Correspondence: Professor B. Boris Vargaftig, Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM 285, 25 Rue du Dr Roux, 75015 Paris, France.

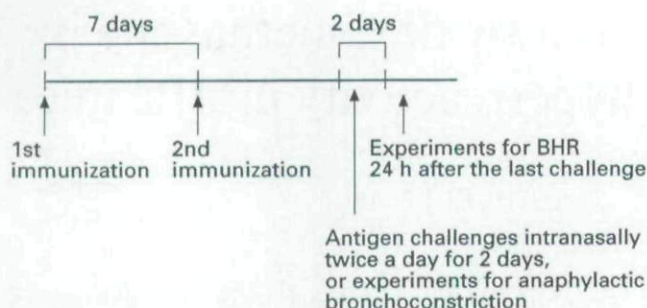


Fig. 1. A schematic diagram of the immunization and antigen provocation protocols of mice.

BHR, using BP2 mice, a selection of Biozzi mice which produce high titres of antibodies, including IgE, after immunization [10]. This murine selection also displays anaphylactic bronchoconstriction when challenged with antigen intravenously.

We demonstrated that the administration of anti-IL-5 antibodies to these mice reduces airways eosinophilia and BHR [10], indicating that IL-5 is involved in the induction of BHR. In view of the relevance of glucocorticosteroids, we investigated the effects of standard dexamethasone on BHR and on the airways eosinophilia in our model as related to IL-5 production.

Materials and methods

Mice

Male BP2 mice were obtained from the Centre d'Elevage R. Janvier (BP5, 53940 Le Genest Saint-Isle, France). They were maintained on standard laboratory chow and water *ad libitum* in the animal facilities of the Institut Pasteur. Mice ranging from 8–11 weeks (30–35 g) were used in the experiments.

Procedures for immunization and antigen challenge

Mice were immunized with 0.4 ml of a solution of 250 µg/ml ovalbumin mixed with 4 mg/ml Al(OH)₃ subcutaneously twice at a 7-day interval. One week after the second injection, the immunized mice were challenged intranasally twice for 2 days (at 09.00 and at 17.00) under light ether anaesthesia with 10 µg ovalbumin in 50 µl of 0.9% NaCl for about 10 s. The control mice were challenged with the same volume of 0.9% NaCl (saline). BHR was evaluated 24 h after the last antigen provocation. To study anaphylactic bronchoconstriction, the immunized mice were anaesthetized as indicated below and challenged intravenously with antigen. The protocols are summarized schematically in Fig. 1.

Evaluation of lung functions

Bronchoconstriction was measured as reported previously [10]. Briefly, mice were placed in a whole body plethysmographic chamber (Buxco Electronics Inc., Sharon, CT), to analyse the respiratory waveforms in unrestrained conscious animals. After stabilization for a few minutes, an aerosol of methacholine (3×10^{-2} M in the aerosolator) was delivered for 20 and 60 sec, at a 10-min interval. The airways resistance was expressed as $\text{Penh} = \text{Te (expiratory time)}/40\% \text{ of Tr (relaxation time)} - 1] \times \text{Pef (peak expiratory flow)}/\text{Pif (peak inspiratory flow)} \times 0.67$. To calculate the ΔPenh (difference between the basal and maximal value), the average of maximal 5 values were used.

After the experiment, the mice were equilibrated in the cage for at least 1 h before the second procedure. The mice were then anaesthetized with ethyl carbamate (*i.p.*, 15 mg/10 g). The trachea was cannulated and prepared for recording of dynamic compliance and airways resistance, by adapting the equipment of the computerized pulmonary analyser (Mumed PR800 system, UK) to mice airways at a tidal volume of 0.2 ml/10 g and a frequency of 100 breaths/min. The animals were paralyzed with 10 µg of pancuronium bromide (Pavulon[®]) intravenously, and airway resistance and dynamic lung compliance were calculated from the differential pressure between the airways and pleural cavity and the airflow.

The effects of antigenic challenge on the airways responsiveness were evaluated by injecting 5-hydroxytryptamine (5-HT) into the cannulated jugular vein at 10, 20, 40, 80, 160, 320 µg/kg in a volume of 100 µl/10 sec at five-minute intervals. Results are expressed as PD30, PD60 and PD90 (the amounts of 5-HT needed to augment the bronchial resistance by 30%, 60%, and 90%). Finally, in separate experiments, ovalbumin was injected intravenously at 50 mg/kg into the immunized and anaesthetized mice in order to induce acute anaphylactic bronchoconstriction. Separate experiments (not shown) have shown that this was the optimal dose.

Bronchoalveolar lavage fluid (BALF) preparations

Immediately after assessing lung function, cells were recovered from the BALF with 8 volumes of 0.5 ml each of 0.9% NaCl. The cell suspension was placed in plastic tubes on ice and the total numbers were counted automatically (Coulter Counter[®] ZM, Coultronics, Margency, France). The cell suspensions were cyto-centrifuged (Hettich Universal, Tuttingen, Germany) and stained according to a May Grunwald Giemsa-derived method (Diff Quick, Baxter Dade AG, Duedingen, Switzerland). Differential cell counts were performed

under a light microscope. Each leukocyte was quantified as a percentage of the total from at least 200 cells and results are expressed as absolute cell numbers of macrophages, eosinophils, lymphocytes and neutrophils in the cell population.

Histological evaluation

The trachea was cannulated under anaesthesia with sodium pentobarbital (i.p., 3 mg/10 g, Sanofi Santé Animal, France) and the thoracic cavity was opened. Lungs were inflated with 1 ml of 10% buffered formaldehyde solution. The lobes were separated and fixed in the same solution for 48 h and later processed for conventional histological studies. After fixation, all lobes were cut perpendicular to their long axis to have a uniform longitudinal section of the lobar bronchus, embedded in paraffin, cut into 4 μ m sections and stained with haematoxylin eosin.

Determination of IL-5 titres

Venous blood was collected from the post vena cava before the bronchoalveolar lavage and placed at room temperature for 30 min to allow coagulation. Serum and the supernatants of BALF were collected after centrifugation for 10 min (500 g for 10 min) and stored at -20°C until use. The levels of IL-5 were determined by enzyme immunoassay. This method involves the reaction of the thiol group of mAb's Fab' fragments with maleimido groups previously introduced into AChE [11]. Maleimido groups were first incorporated into AChE with the heterobifunctional reagent N-succinimidyl-4(N-maleimido-methyl)-cyclohexane-1-carboxylate. TRFK-5 and TRFK-4 antibodies were purified from ascitic fluids (cloned hybridomas kindly provided by Dr Paola Minoprio, Institut Pasteur, Paris, France), using the affinity chromatography method on a protein G column (HiTrap affinity columns, Pharmacia Biotechnology, Uppsala, Sweden) after precipitation by ammonium sulfate. The characteristics of these antibodies are described in detail elsewhere [12].

The purity of this preparation was assessed by polyacrylamide gel electrophoresis techniques in denaturing and non-reducing conditions. F(ab')₂ fragments were obtained from purified antibodies by treatment with pepsin in acidic medium [13]. Fab' fragments were further obtained from F(ab')₂ by reduction in the presence of 2-mercaptoethanolamine. The thiol content of the Fab' preparation was determined colorimetrically after reaction with 3,5'-dithiobis-notrobenzoic acid, as described by Ellman [14].

Immunoassays were performed in 96-well

microtitre plates (MaxiSorp, Nunc, Roskilde Denmark), coated with 10 μ g/ml of the rat monoclonal antibody for murine IL-5 (TRFK-4) as described previously [15]. The one-step procedure used for immunoassays involved the simultaneous addition of 100 μ l of the IL-5 standards (7.8–1000 pg/ml) or experimental samples, and 100 μ l of the second rat anti-IL-5 antibody, TRFK-5 conjugated to AChE, at the concentration of 10 Ellman units/ml. After incubation for 18 h at 4°C , the plates were extensively washed and solid-phase bound AChE activity was determined colorimetrically by the addition of 200 μ l of Ellman's medium [16]. Absorbance was read at 405 nm with an automatic microplate reader (Dinatech MR 5000; Dinatech Laboratories, Saint Cloud, France). The lower limit of detection of this assay is approximately 5 pg IL-5/ml sample.

Protocols of dexamethasone administration

Dexamethasone phosphate in 0.9% NaCl was injected i.p. into immunized BP2 mice at 5 mg/kg, 1 h before the first of the four antigen challenges (indicated as Dexa. II), or at 1.25 mg/kg 1 h before each of the four challenges (indicated as Dexa. I). BHR was evaluated 24 h after the last antigen challenge.

To study its effects on anaphylactic bronchoconstriction, dexamethasone was administered i.p. at 5 mg/kg to the immunized mice either 1–3 h or 14–17 h before injecting ovalbumin into the cannulated jugular vein at 50 mg/kg in 0.1 ml. Results are presented as per cent augmentation of the bronchial resistance as compared to the basal values.

Materials

Ovalbumin (5 $\times$ crystallized) was from Immunobiological (Costa Mesa, USA). Aluminium hydroxide was from Merck (Darmstadt, Germany). 5-Hydroxytryptamine (5-HT), ethyl carbamate and dexamethasone phosphate were from Sigma (St Louis, MO, USA). Methacholine (acetyl- β -methylcholine chloride) was from Aldrich-Chemie (Steinheim, Germany). Pancuronium bromide was from Organon Teknika (France). Other materials are indicated under 'Methods'.

Statistical analysis

The results of each measurement are reported as mean $\pm$ standard error of the mean. The significance of differences between the experimental and control groups was examined by the unpaired Student's *t*-test, and *P* values of less than 0.05 were considered to be significant.

Results

Effect of dexamethasone on the eosinophil recruitment in the BALF

In saline-treated and saline-challenged control immunized mice, the BALF contained $1.41 \pm 0.17 \times 10^5$ cells/ml. Dexamethasone-treated and saline-challenged mice contained comparable cell numbers ($1.08 \pm 0.08 \times 10^5$ cells/ml) (Fig. 2 and Table 1). Few eosinophils (below 1% of total cells) were found in both control groups. Ovalbumin challenges augmented the total cell numbers up to $3.16 \pm 0.55 \times 10^5$ ($P < 0.01$), an increment accounted for by a marked increase in eosinophil counts (55.0 $\pm$ 2.79% of total cells) (Fig. 2 and Table 1). The numbers of neutrophils did not change significantly with ovalbumin challenges (Table 1).

When mice were treated with dexamethasone once at 5 mg/kg 1 h before the first of the four ovalbumin challenges (Dexa II), the total cell and eosinophil numbers in the BALF were reduced, but not significantly, compared to saline-challenged animals (Fig. 2 and Table 1). Since mice were provoked for 2 days and analysed 48 h after the injection of dexamethasone, its protective effects might be over. Mice were thus injected four times with 1.25 mg/kg of dexamethasone, 1 h before each intranasal provocation (Dexa I). In this case, total BALF cell and eosinophil numbers were completely suppressed (from $1.79 \pm 0.39 \times 10^5$ to $0.03 \pm 0.01 \times 10^5$ cells/ml) (Fig. 2 and Table 1).

Effect of dexamethasone on bronchial responsiveness

The baseline values of bronchial resistance were increased by antigen challenges, since saline-challenged

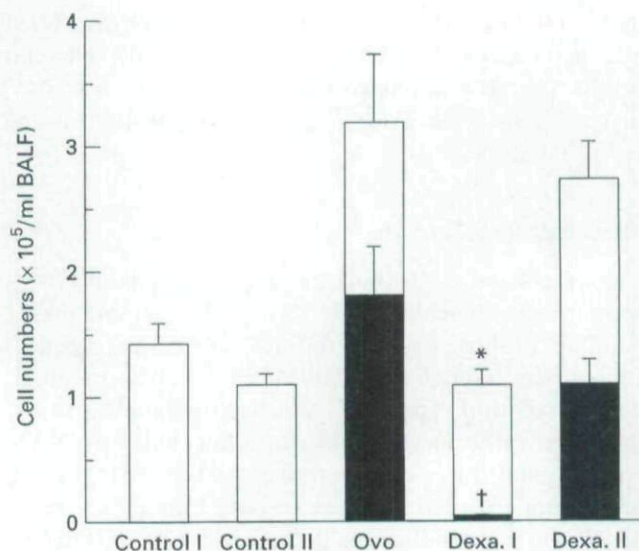


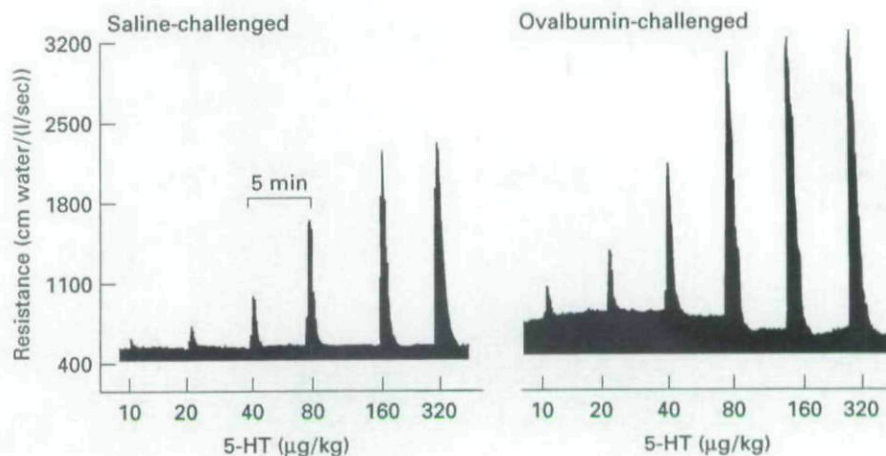
Fig. 2. Effect of dexamethasone on the recruitment of eosinophils into the BALF after ovalbumin challenges. Dexamethasone was injected four times at 1.25 mg/kg each (dexa. I) or at 5 mg/kg once only (dexa. II), before ovalbumin challenges. The group 'Ovo' was treated with saline and challenged with ovalbumin. Control animals were challenged with saline in two groups, a saline-treated group (control I) and a dexa. I-treated group (control II). The total BALF cell (□) and eosinophil (■) numbers of the group 'Ovo' were significantly augmented ($P < 0.01$ and $P < 0.001$, respectively) compared to controls. The statistical comparison was performed for the groups 'Dexa. I or Dexa. II' and compared to the group 'Ovo'. Each value represents mean $\pm$ SEM of 4–9 mice and significant differences are indicated: * $P < 0.002$; † $P < 0.001$.

Table 1. Cell composition of the BALF of immunized mice after antigen challenges

Treatment	Challenge	BALF cells ($\times 10^{-5}$ /ml)				
		Total cells	Macro.	Eosino.	Neutro.	Lympho.
Saline	Saline	1.41 (0.18)	1.18 (0.16)	0	0.22 (0.02)	0.006 (0.00)
Dexa. I	Saline	1.08 (0.08)	0.91 (0.03)	0	0.16 (0.07)	0.004 (0.00)
Saline	Ovalbumin	3.16 (0.55)	0.96 (0.11)	1.79 (0.39)	0.30 (0.06)	0.09 (0.02)
Dexa. I	Ovalbumin	1.07 (0.13)*	0.87 (0.12)	0.03 (0.01)†	0.16 (0.05)	0.004 (0.00)†
Dexa. II	Ovalbumin	2.69 (0.30)	0.73 (0.06)	1.07 (0.21)	0.81 (0.11)	0.07 (0.02)

All mice were tested 24 h after the last of four challenges. In saline-treated ovalbumin-challenged mice, total BALF cell ($P < 0.01$), and eosinophils ($P < 0.001$), and lymphocytes ($P < 0.05$) numbers were significantly augmented as compared to saline-treated and saline-challenged mice. Protocols for dexamethasone treatment are explained in 'Methods' and in the legend for Figure 1. Data are absolute cell numbers for 4–9 mice and SEM are indicated in parentheses. Significant differences between dexamethasone-treated and ovalbumin-challenged mice, on one side, and saline-treated and ovalbumin-challenged mice, on the other, are indicated: * $P < 0.002$; † $P < 0.001$.

Fig. 3. Typical tracing of bronchoconstriction in response to i.v. 5-HT in immunized and saline-challenged mice (left) and immunized and ovalbumin-challenged mice (right). Serotonin was injected every 5 min and the bronchoconstrictor responses were more intense in ovalbumin-challenged mice.



mice had a value of 466 ± 9 cm water/(l/sec), whereas ovalbumin-challenged mice had a value of 708 ± 24 cm water/(l/sec) ($P < 0.001$). Both protocols of administration of dexamethasone protected against this increase, since the values for the Dexa I ($P < 0.001$) and for the Dexa II ($P < 0.01$) group were 489 ± 32 and 570 ± 29 cm water/(l/sec), respectively.

Bronchoconstriction in response to i.v. 5-HT was significantly augmented in ovalbumin-challenged mice, as compared to saline-challenged mice (Figs 3 and 4).

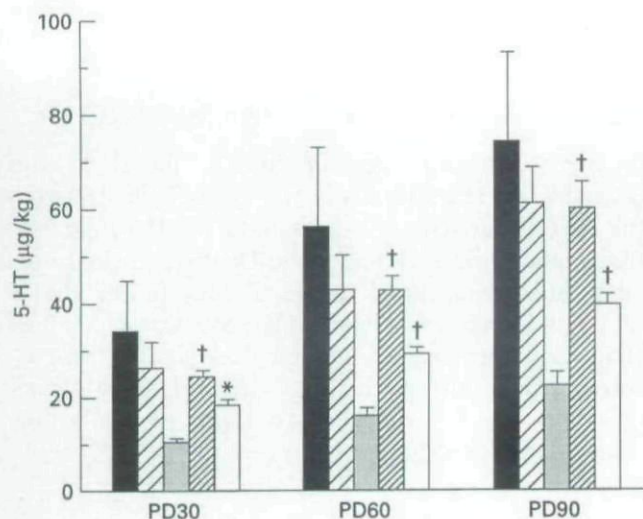


Fig. 4. Effects of dexamethasone on the bronchial responsiveness of anaesthetized mice in response to 5-HT i.v. after antigen challenges. Ovalbumin-challenged mice (■) showed a significant augmentation ($P < 0.01$ for PD30, 60 and 90) of bronchoconstriction compared to controls I (■) and II (▨). The results of dexa. groups I (▨) and II (□) were compared with saline-treated ovalbumin-challenged mice. Each value represents mean $\pm$ SEM of 4–9 mice and significant differences are indicated: * $P < 0.005$ and † $P < 0.001$.

Administration of dexamethasone using both protocols before ovalbumin challenges, significantly reduced ($P < 0.01$) bronchoconstriction (Fig. 4), but the protocol in which dexamethasone was injected four times at 1.25 mg/kg was more effective.

In non-anaesthetized mice, BHR was also uncovered when ovalbumin-challenged mice were exposed to aerosolized methacholine (Fig. 5). Dexamethasone at 5 mg/kg

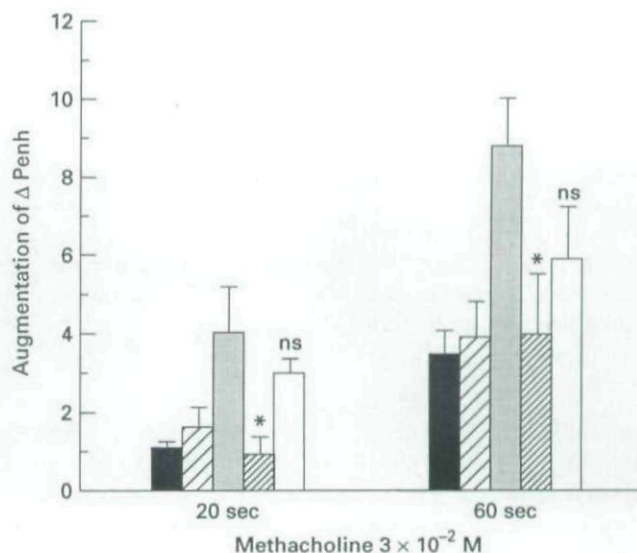


Fig. 5. Effects of dexamethasone on the bronchial responsiveness of non-anaesthetized mice in response to inhaled methacholine, after antigen challenges. Bronchial resistance of ovalbumin-challenged mice (■) was augmented significantly ($P < 0.05$ for 20 sec and $P < 0.01$ for 60 sec) compared to controls I (■) and II (▨). The results of dexa. groups I (▨) and II (□) were compared with saline-treated and ovalbumin-challenged mice. Each value represents mean $\pm$ SEM of 4–9 mice. Significant differences are indicated (* $P < 0.05$) and non-significances are expressed as ns.

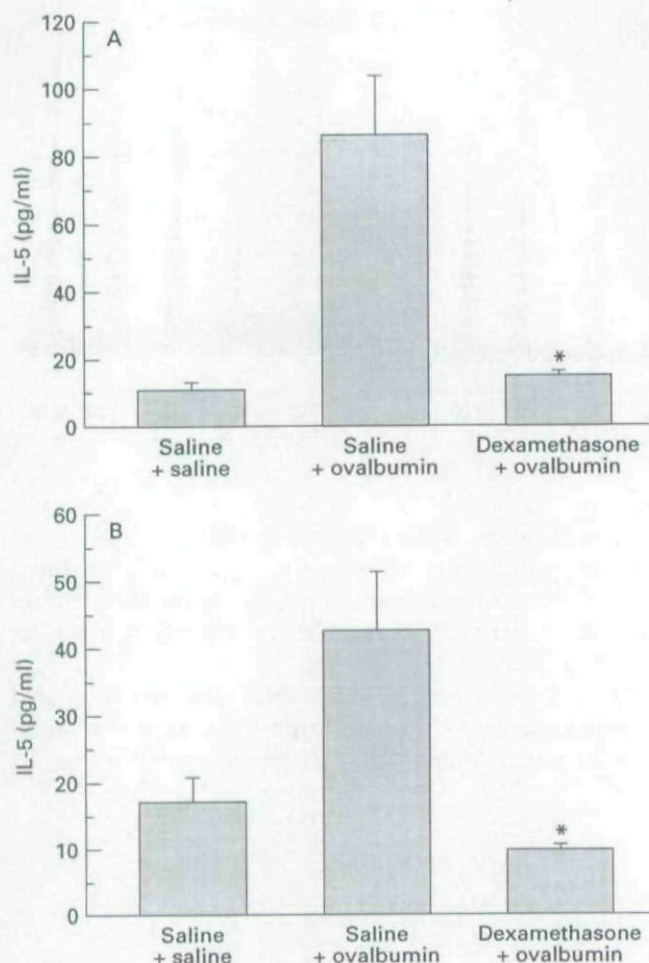


Fig. 6. Effects of dexamethasone on the IL-5 titres in the BALF (A) and in the serum (B). Dexamethasone was administered at 1.25 mg/kg i.p. 1 h before each antigen provocation. The BALF and serum were taken at 3–6 h after the last antigen challenge. Each value represents mean $\pm$ SEM of 4–13 mice and significant differences are indicated: * $P < 0.05$.

once only before the first of the four ovalbumin challenges did not significantly reduce the enhanced responsiveness to methacholine, but its administration using the protocol of 4×1.25 mg/kg before each antigen provocation suppressed BHR (Fig. 5).

Effect of dexamethasone on histology

No eosinophils, nor other inflammatory cells were found in the lungs of saline-challenged mice. After ovalbumin challenges, many eosinophils and lymphocytes infiltrated the peribronchial and perivascular tissues. When the mice were treated with dexamethasone at 1.25 mg/kg four times before each antigen challenge,

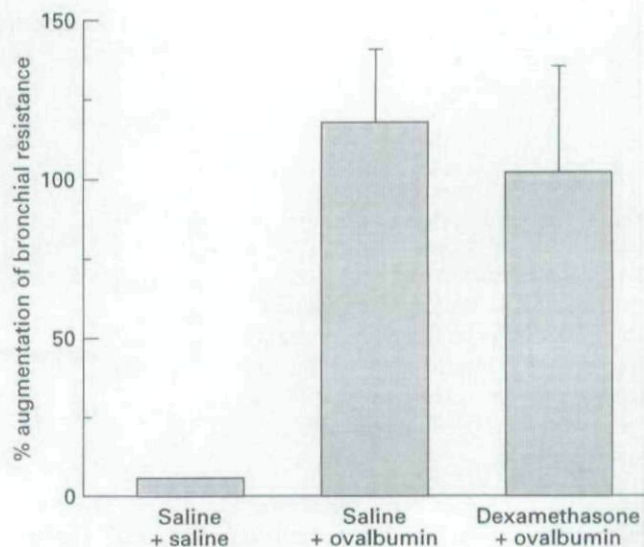


Fig. 7. Effect of dexamethasone on the anaphylactic bronchoconstriction. Mice were immunized and ovalbumin was injected at 50 mg/kg i.v. in a volume of 100 μ l. For the control mice, the same volume of saline was injected. Dexamethasone was administered at 5 mg/kg i.p. and ovalbumin was injected at 1–3 h or 14–17 h after administration. Dexamethasone (dexa. + ovo) failed to inhibit bronchoconstriction. Each value represents mean $\pm$ SEM of 8–9 mice.

inflammation cell numbers were markedly reduced (results not shown).

Effect of dexamethasone on the production of IL-5

In the preliminary study of kinetics, the IL-5 titres peaked 3–6 h after the last of four antigen challenges in the BALF and serum. The recovery of IL-5 titres was thus evaluated at this time point. Ovalbumin challenges augmented significantly the IL-5 titres in the BALF ($P < 0.001$) and serum ($P < 0.05$), compared to saline-challenged mice (Fig. 6). Dexamethasone at 1.25 mg/kg before each antigen challenge reduced these values ($P < 0.05$) to levels similar to those of the saline-challenged group (Fig. 6).

Failure of dexamethasone to reduce anaphylactic bronchoconstriction

As shown in Fig. 7, the i.v. ovalbumin challenge at 50 mg/kg to immunized mice, induced an intense bronchoconstriction (about 120% augmentation of bronchial resistance), compared to saline-injected mice (below 5% of the augmentation). This augmentation started to show by 10 sec after the injection and was over within 5–10 min. Mice were treated once with

dexamethasone at 5 mg/kg. Ovalbumin injected i.v. 1–3 h or 14–17 h later induced bronchoconstriction comparable to that in saline-treated immunized controls (Fig. 7).

Discussion

There is overwhelming evidence that inflammatory cells infiltrating the bronchial mucosa of patients with bronchial asthma participate in the mechanisms leading to airways obstruction and BHR [1,17–19]. In our previous study, we confirmed that BHR develops in association with eosinophilic inflammation of the bronchial epithelium and of the submucosa of antigen-challenged BP2 mice [10]. This is very important, because the mouse is well characterized from an immunologic standpoint. The availability of mutants and inbred strains displaying immunologic deficiencies also provides unique opportunities to study the role of single compartments of the immune system. These BP2 mice are selected from a high responder line of Biozzi mice by a bi-directional breeding method for antibody responsiveness to sheep erythrocytes in terms of agglutinin titres [20]. Both BHR and airways eosinophilia were fully inhibited by an antibody against IL-5, a cytokine mainly released by Th2 lymphocytes [10].

Glucocorticosteroids are very potent immunosuppressive agents and are widely used to treat inflammatory diseases, including chronic asthma. The clinical improvement by glucocorticosteroids in asthma is associated with the reduction of lung eosinophils and T lymphocytes [8]. In the present study, dexamethasone suppressed eosinophilia in the airways and in the BALF, as well as BHR induced in BP2 mice by repeated antigen challenges. The IL-5 titres in the BALF and serum were concomitantly reduced by dexamethasone. IL-5 is the main eosinophil-active cytokine present in BALF during allergen-induced late-phase inflammation [21] and is involved in BHR [10,22].

The ability of glucocorticoids to inhibit the release or the effects of cytokines is likely to be of great importance in the treatment of asthma. Dexamethasone is a potent inhibitor of T-cell proliferation induced by antigen or mitogens [23] and is very effective in inhibiting the production by human T cells of IL-2, interferon- γ (INF- γ) and IL-4 [24–26]. Further, it has been shown that dexamethasone inhibits IL-5 gene expression in human peripheral blood mononuclear cells [27]. The inhalation of beclomethasone by asthmatic patients ameliorates the clinical symptoms, improves lung functions, and markedly suppresses IL-5 production by peripheral blood mononuclear cells [9]. In our study, dexamethasone indeed inhibited the IL-5 titres in blood and BALF.

Lefort *et al.* [28] demonstrated that a single injection of dexamethasone at 5 mg/kg decreases BALF eosinophilia and suppresses the lung inflammation which follows a single antigen challenge in immunized Swiss mice. In the present study, two protocols of dexamethasone administration were used. First, one dose of 5 mg/kg was administered 1 h before the first of the four antigen challenges. In this case, the total BALF cell and eosinophil numbers were not significantly reduced and BHR was also partially inhibited. By contrast, when the dose of dexamethasone was divided into four fractions, which were delivered at 1.25 mg/kg four times, 1 h before each of the four antigen challenges, airways eosinophilia and BHR were completely suppressed. These results suggest that the last provocations were performed at a time when the protective effects of dexamethasone were over and show that persistent immune stimulation requires constant treatment.

In general, mice do not develop anaphylactic bronchoconstriction upon allergen challenge [29,30], but immunized BP2 mice displayed a potent bronchoconstrictor effect in response to i.v. ovalbumin, which was completely blocked by the serotonin antagonist methysergide. It is widely believed that peptide-leukotrienes play an important role in immediate bronchoconstriction following antigen challenge. Lipoxigenase inhibitors reduce this acute bronchoconstriction and the late-phase lung eosinophil accumulation in guinea-pigs [31] as well as BHR in humans [32]. In our model, however, the lipoxigenase inhibitor MK-571 failed to modify anaphylactic bronchoconstriction. This observation suggests that peptido-leukotrienes are not essential for anaphylactic bronchoconstriction in BP2 mice. Our results indicate an important role for serotonin, likely to be released from mast cells, the major cell type responsible for immediate hypersensitivity and anaphylactic shock [33,34]. Glucocorticoids generally do not inhibit the release of histamine or of leukotrienes from the human mast cells [35,36], but do inhibit mediator release from murine [37], rat [38] and guinea pig [39] mast cells. Thus, dexamethasone inhibits allergen-induced acute bronchoconstriction in rats [40] and in guinea-pigs [39]. Nevertheless, in our study dexamethasone failed to inhibit anaphylactic bronchoconstriction. Daeron *et al.* [37] have demonstrated that pretreatment of mouse peritoneal mast cells *in vitro* with dexamethasone resulted in inhibition of antigen-induced histamine release and degranulation, and that this effect was increased linearly with the duration of the treatment, maximal inhibition being obtained after approximately 16 h with dexamethasone. In the present study, ovalbumin was injected 1–3 h or 14–17 h after treatment with dexamethasone, but no inhibitory effect was detected. Since

dexamethasone inhibits murine mast cell degranulation *in vitro* [37], its failure to modify the serotonin-mediated bronchoconstriction may point to another source for serotonin in anaphylactic bronchoconstriction. Platelets are the likely candidates, and are presently being investigated. In summary, dexamethasone inhibited allergen-induced lung inflammation and the production of IL-5 in the BALF and serum. Histological observation confirmed the reduction of the infiltration of eosinophils and lymphocytes in the bronchial wall after dexamethasone administration. As a consequence, BHR was suppressed. These results suggest that glucocorticoids exerted their anti-inflammatory effects in the murine model by inhibiting the formation of IL-5. Since dexamethasone failed to inhibit anaphylactic bronchoconstriction, it is clear that allergic eosinophilia and BHR, on one side, and acute bronchial anaphylaxis, on the other, are distinct mechanisms, which may be modulated separately.

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