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Fluticasone Propionate and Pentamidine Isethionate Reduce Airway Hyperreactivity, Pulmonary Eosinophilia and Pulmonary Dendritic Cell Response in a Guinea Pig Model of Asthma[1]

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ABSTRACT

In this study, we examined the effects of fluticasone propionate (FP) and pentamidine isethionate (PI) on antigen-induced lung inflammation and airway hyperreactivity in guinea pigs. Male guinea pigs were sensitized on days 0 and 14 with 10 μg of ovalbumin (OVA) plus 1 mg of $Al(OH)_3$. On day 21, animals were challenged with a 2% OVA aerosol inhalation until they developed pulmonary obstruction. Animals were treated with aerosol inhalation of FP (2 ml of 0.5 mg/ml, five consecutive doses at 12-hr intervals with the last dose given 6 hr before OVA challenge) or PI (30 mg/ml for 30 min 1 hr before OVA challenge), and control animals received no drug before OVA challenge. Airway reactivity to methacholine (MCh) was assessed before sensitization and 18 hr after OVA challenge. At 18 hr after challenge, histological sections of trachea and lung were examined for eosinophil, dendritic cell (DC) and macrophage cell densities in the airways. In control animals, OVA evoked airway hyperreactivity to MCh in conjunction with pulmonary eosinophilia and increases in DC prevalence in the trachea and bronchi. Treatment with FP or PI abolished the OVA-induced hyperresponsiveness and significantly reduced the OVA-induced increases in eosinophils and DCs in the airways. FP and PI had no effect on saline-treated animals. Our study indicates that both inhaled FP and inhaled PI reduce antigen-induced airway hyperreactivity and pulmonary inflammation in guinea pigs. The results also suggest that the DC is a target of the anti-inflammatory effects of these drugs in the airways.

Asthma is a common disease that is estimated to affect as many as 12 million persons in the United States alone (Weiss *et al.*, 1992). It is characterized by episodes of reversible airway obstruction, increased mucus production and infiltration of eosinophils and mononuclear cells into the airways (Corrigan and Kay, 1991; Sears, 1993). One particular form of the disease, allergen-induced asthma, is linked to airway hyperresponsiveness (Busse and Sedgwick, 1992). Patients with allergen-induced asthma exhibit an immediate or early-phase response that is characterized by the abrupt onset of bronchoconstriction. A second obstructive response, the late-phase response, occurs 8 to 24 hr after allergen exposure in conjunction with inflammation in the airways and airway hyperresponsiveness.

This late-phase allergic response is associated with the influx of eosinophils into the airways (Busse and Sedgwick, 1992). The release of several toxic proteins, leukotrienes and free radicals from activated eosinophils are capable of producing significant lung tissue injury (Frigas *et al.*, 1980, 1991). It has been shown that the severity of asthma can be correlated to the degree of inflammation in the airways, in which eosinophils are the predominant inflammatory cell type (Frew and Kay, 1990). Inflammation in response to allergen exposure is dependent on the presentation of the allergen for T lymphocyte activation (Hamacher and Schaberg, 1994). In the lung, there are three types of accessory cells capable of antigen presentation: alveolar macrophages (Holt and Batty, 1980; Pretolani *et al.*, 1988), B lymphocytes (Kammer and Unanue, 1980) and DCs (Steinman and Nussenzweig, 1980; Sunshine *et al.*, 1980).

DCs are hemapoietic cells derived from the same lineage as epidermal Langerhans cells (Schuler and Steinman, 1985; Tew *et al.*, 1982). They are characterized by their long, slender processes (Steinman and Nussenzweig, 1980) and high MHC class II expression (Steinman, 1991). The interaction of the MHC class II molecule with the T cell receptor, as well as the B7/CTLA-4 costimulatory pathway, is highly important in the initiation and maintenance of the allergen-induced inflammatory cascade (Reiser and Stadecker, 1996).

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ABBREVIATIONS: BF, breathing frequency; DAB, diaminobenzidine; DC, dendritic cells; FP, fluticasone propionate; OVA, ovalbumin; PI, pentamidine isethionate; MCh, methacholine; SRaw, specific airway resistance.

Immunohistochemical analysis of guinea pig bronchi at 2 and 24 hr after intraperitoneal OVA challenge of subcutaneous OVA-sensitized animals revealed no increases in eosinophils, T lymphocytes or DC at 2 hr. However, at 24 hr, significant influxes of eosinophils and MHC class II-positive cells were seen, whereas the number of T lymphocytes remained unchanged (Lapa e Silva *et al.*, 1994). These data suggest a possible role for DC in the maintenance of airway inflammation after antigen presentation. In a recent study by Keane-Myers *et al.* (1997), it was demonstrated that administration of CTLA-4-Ig in a murine model of asthma resulted in the abolition of airway hyperresponsiveness and pulmonary eosinophilia produced by allergen challenge, suggesting that the interaction between DC and T lymphocytes is critical for the development of airway inflammation.

Mast cells have been suggested to play a significant role in allergen-induced eosinophil recruitment (Kung *et al.*, 1995) independent of the involvement of antigen presenting cells. However, Hom and Estridge (1994) have shown in mast cell-deficient mice a significant eosinophil migration into the airways in response to allergen challenge. In fact, products of activated mast cells in culture (de Pater-Huijsen *et al.*, 1997) produced proliferation of $CD8^+$ T cell clones, suggesting that mast cells may decrease Th2-type responses seen in asthma through increases in a $CD8^+$-predominant Th1-type response. Thus, although many different cell types take part in allergen-induced inflammation, the activation of T cells by antigen presenting cells, such as DCs, are integral to the initiation and maintenance of an inflammatory response.

The purpose of this investigation was to assess the effects of two anti-inflammatory compounds, FP and PI, on airway inflammation and airway hyperreactivity in the late-phase response of guinea pigs that were sensitized and challenged *via* inhalation. Specifically, we wanted to characterize the relationship among pulmonary DC prevalence, airway inflammation and hyperreactivity and the ability of the drugs to inhibit the development of these responses. The hypothesis of this study was that DCs play a central role in stimulating antigen-induced lung inflammation and airway hyperreactivity in asthma. The significance of such a role is that DCs could represent new cellular targets for pharmacological manipulation to reduce the obstructive responses that accompany the onset and exacerbation of asthma.

FP and PI were chosen for study because they exert anti-inflammatory effects by two different mechanisms. By far, the most effective treatment for patients with asthma is the administration of glucocorticoids (Cronstein and Weissmann, 1995). Administration of one glucocorticoid, budesonide, twice a day for 3 months has been shown to significantly reduce base-line airway obstruction, airway hyperresponsiveness to histamine, and the total number of T lymphocytes and $RFD1^+$ macrophages and HLA-DR expression in bronchial biopsies (Burke *et al.*, 1992). Glucocorticoids bind to cytosolic glucocorticoid receptors, and the glucocorticoid-receptor complexes translocate to the nucleus. The steroid/receptor complexes bind to the glucocorticoid-responsiveness elements of genes to either increase or decrease transcription (Barnes, 1995) involved in the regulation of gene expression for inflammatory cytokines (Ray and Prefontaine, 1994). FP is currently used in the United States as a nasal spray for the treatment of allergic rhinitis. In clinical trials, FP has been shown to have greater anti-inflammatory potency in asthmatics than other inhaled steroids, such as beclomethasone and budesonide (Holliday *et al.*, 1994).

Although currently not indicated for the management of asthma, PI is a nonsteroidal aromatic diamidine that is used for the treatment of *Pneumocystis carinii* pneumonia in patients with AIDS (Wispelwey and Pearson, 1991). Its mechanism of action is not certain. *In vitro*, PI has been shown to reduce tumor necrosis factor-α (Corsini *et al.*, 1992) and interleukin-1 (Rosenthal *et al.*, 1991) release from alveolar macrophages and inhibit B lymphocyte responses (Ferrante and Secker, 1985). *In vivo*, PI inhibits contact hypersensitivity through a decrease in MHC class II-positive Langerhans cells (Blaylock *et al.*, 1991) and blocks the pathophysiological effects of endotoxemia through inhibition of tumor necrosis factor-α and interleukin-6 release (Rosenthal *et al.*, 1992). These studies suggest that PI possesses an anti-inflammatory action through the inhibition of cytokine release. Such effects, were they to occur in the lungs, could reduce the late-phase response.

Methods

OVA sensitization and challenge protocols. Male Dunkin-Hartley guinea pigs (Harlan, Indianapolis, IN) (300–350 g) were sensitized and challenged with OVA (Sigma Chemical, St. Louis, MO) as follows: animals were sensitized by subcutaneous injection of 0.5 ml of 0.9% NaCl containing 10 μg of OVA dispersed in 1 mg of $Al(OH)_3$ in the nuchal region on days 0 and 14. On day 21, the animals were challenged with OVA aerosol (2% dissolved in saline) until an obstructive airway response appeared. The onset of obstruction was indicated on the basis of increases in BF and SRaw (see below). Control animals received saline aerosol for 3 min on day 21.

Aerosols were generated with an Ultra Neb 99 Devilbiss nebulizer (Devilbiss, Somerset, PA). The details of the method for aerosol generation and regulation of the aerosols have been described previously (Lawrence *et al.*, 1997).

Pulmonary function measurements. Basal SRaw and BF were measured in conscious guinea pigs 1 min before OVA challenge, 1 min after challenge and 18 hr after challenge using a two-chamber whole-body plethysmograph (Buxco Electronics, Troy, NY) connected to a noninvasive airway mechanics analyzer (model LS-20; Buxco). SRaw and BF data were logged at 6-sec intervals. The mean of 10 consecutive interval averages was calculated as the measurement at each time point. BF was calculated as the number of breaths taken in 1 min (BPM), and SRaw was calculated as airway resistance multiplied by the thoracic gas volume (cm $H_2O \cdot$ sec).

Lung removal and fixation. At 18 hr after OVA challenge, guinea pigs were anesthetized intraperitoneally with sodium pentobarbital (The Butler Co., Columbus, OH; 50 mg/kg) and exsanguinated by severing the abdominal aorta. A 3-cm length of trachea was marked, and an incision was made in the lower trachea through which a 1-cm piece of polyethylene tubing placed on a 15-gauge needle was inserted and tied. The trachea was then dissected out and stretched to 3 cm. The lungs were inflated with Histocon (Polysciences, Warrington, PA) at a pressure of 30 cm H_2O and removed *en bloc*. The trachea and right diaphragmatic and left cardiac lobes of the lung were snap-frozen in isopentane cooled with liquid nitrogen. The tracheal sections were cut tangentially parallel to the longitudinal axis; lung lobes were cut so the largest airways were included in the sections. Tissue sections, 6 μm thick (Hacker-Bright Micro Cryostat 2122; Hacker Instruments, Fairfield, NJ), were collected on Vectabond-coated slides (Vector Laboratories, Burlingame, CA) and air-dried.

Immunohistochemistry: tissue macrophages and DCs. Tissue macrophage prevalence and distribution were identified after incubation of sections with the anti-guinea pig monoclonal antibody

MR-1 (Harlan Bioproducts for Science, Indianapolis, IN), a marker for phagocytic tissue macrophages (Kraal *et al.*, 1988).

Because there are no available antibodies specific for guinea pig DCs, the cells were characterized according to the following criteria: (1) positive staining with the anti-guinea pig monoclonal antibody CL.13.1 (Harlan Bioproducts for Science), a marker for MHC class II cells (*i.e.*, macrophages and DCs), (2) negative staining with MR-1, a marker for phagocytic tissue macrophages and (3) dendritic morphology.

Consecutive sections of the lung and trachea were incubated for 30 min with 200 μl of each of the monoclonal antibodies described previously. The primary antibodies were localized by utilization of alkaline phosphatase anti-alkaline phosphatase staining technique (DAKO Kit K670; Dako, Carpinteria, CA) (Cordell *et al.*, 1984), as described previously (Lawrence *et al.*, 1997).

Eosinophil detection. A histochemical method for cyanide-resistant eosinophil peroxidase activity was used to stain for eosinophils present within the respiratory tissue (Yam *et al.*, 1971; Zucker-Franklin and Grusky, 1976).

Slides were incubated in 0.5 mg/ml DAB containing 0.015% H_2O_2 and 0.01M KCN in Tris-buffered saline, pH 7.6, for 5 min at room temperature. After two consecutive washes with PBS, sections were counterstained with Harris hematoxylin (Polysciences, Warrington, PA).

***In vivo* reactivity to MCh.** Changes in reactivity to MCh were assessed by measuring SRaw in conscious animals before sensitization and 18 hr after OVA challenge. Each guinea pig was placed in the plethysmograph box for a 10-min acclimation period. An aerosol of saline followed by increasing concentrations of MCh (Sigma) from 0.1 to 80 mg/ml in increments of 2-fold were given for 3 min *via* a side port in the head chamber of the plethysmograph. SRaw readings were taken at 6-sec intervals for 1 min after each exposure. Increasing MCh concentrations were given until SRaw was approximately three times the base-line reading measured after delivery of saline aerosol to the animal.

Administration of drugs. FP (a gift from Glaxo, Hertfordshire, UK) was prepared as a fine suspension in 0.1% ethanol. FP was given as aerosol (0.5 mg/ml) for 10 min five times at 12-hr intervals. The last FP dose was given 6 hr before OVA challenge. This dosing regimen was based on a study on the effects of aerosolized budesonide on inflammation and airway hyperreactivity in trimellitic anhydride-sensitized guinea pigs (Hayes *et al.*, 1993).

PI (a gift from Armour Pharmaceutical, Collegeville, PA) was prepared in distilled water. PI was given as aerosol (30 mg/ml) for 30 min at 1 hr before OVA challenge. This dosing regimen was based on the outcome of a study of the tissue distribution of aerosolized PI in rats (Debs *et al.*, 1987) and preliminary studies performed in our laboratory (data not shown).

After drug administration, groups of animals were assessed for *in vivo* changes in airway reactivity to MCh or for inflammatory cells 18 hr after OVA challenge, as described previously.

Treatment effects on SRaw: Quantitation and statistics. All data are expressed as arithmetic mean ± S.E.M. A value of $P < .05$ was considered significant. The effects of treatment on SRaw were analyzed using one-way analysis of variance with repeated measures.

To characterize reactivity to inhaled MCh, SRaw values logged for each MCh concentration were averaged and plotted *vs.* the MCh concentration. The MCh concentration that doubled SRaw ([MCh] PC_{200}) above the basal level was determined by linear interpolation from concentration-response curves. Changes in reactivity to MCh were analyzed with a paired *t* test.

Immunohistochemistry and enzyme histochemistry: Quantitation and statistics. Slides were coded and read in a blinded fashion. Positive cells in the trachea were enumerated in several regions: the epithelium between the lumen and basement membrane, lamina propria between the basement membrane and smooth muscle and the submucosa between the smooth muscle and cartilage. Positive cells in the bronchi were enumerated in the lamina propria, between the basement membrane and the smooth muscle, and in the adventitia, outside the smooth muscle (Bai and Prasad, 1994). Cells present in the epithelium of the bronchi could not be quantified due to the extensive folding of the epithelial layer. The number of inflammatory cells in the areas of interest was determined as described previously (Lawrence *et al.*, 1997) using the Optimas Image Analysis System (Optimas Corp., Edmonds, WA). Results are expressed as the number of positive cells/mm^2 compartment area (mean ± S.E.M.).

Eosinophils were not enumerated in the bronchial epithelium due to extensive epithelial folding and because the large eosinophilic influx into the epithelium after OVA treatment made it difficult to distinguish individual cells; therefore, the eosinophils were quantified only in the trachea. Image analysis was used to calculate the percentage of the area of the epithelium that was DAB positive; we designate this percentage as the Eosinophil Influx Index.

Treatment effects on inflammatory cell prevalence in different regions of the airways were examined for significance using the Mann-Whitney rank sum test. The effects of treatment on airway eosinophils were determined by one-way analysis of variance with *post-hoc* comparisons by the Student-Newman-Keuls test or the unpaired *t* test, where indicated.

Results

Effects of FP on obstruction, inflammation and airway hyperreactivity in response to OVA challenge. Treatment with FP before OVA challenge did not inhibit the increase in SRaw that is observed immediately after OVA challenge (Lawrence *et al.*, 1997).

At 18 hr after challenge, significant inhibitory effects on the inflammatory response were observed. FP induced a 70% decrease in the OVA-induced influx of eosinophils into the tracheal epithelium (fig. 1). This FP-induced reduction in OVA-treated animals was not different from the eosinophil influx in saline-treated and saline plus FP-treated animals (fig. 1).

FP administration did not affect macrophage cell number in the tracheal epithelium of saline- or OVA-treated animals (data not shown).

OVA challenge also evoked an increase in DC prevalence. The increase in DC number was significantly inhibited by FP treatment in the trachea by 56%, 70% and 104% in the epithelium, lamina propria and submucosa, respectively (fig. 2). FP reduced DC prevalence in the tracheal epithelium and submucosa to a level that was not different from that of

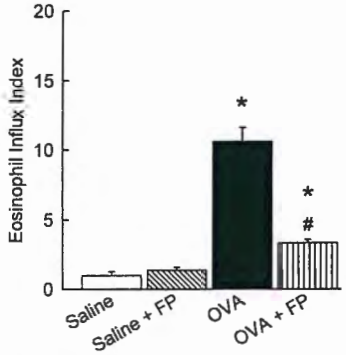

Fig. 1. Effect of FP treatment on eosinophil prevalence in tracheal epithelium 18 hr after saline or OVA challenge. The Eosinophil Influx Index represents the percentage of the epithelial area that was DAB positive. $n = 7$ or 8 for OVA-treated animals, $n = 4$ for saline-treated animals. * $P < .05$ compared with saline-treated animals; # $P < .05$, OVA *vs.* OVA + FP.

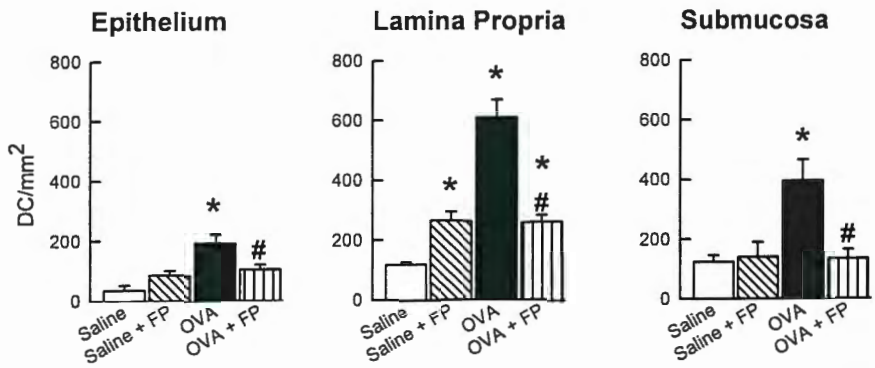

Fig. 2. Effect of FP treatment on DC prevalence in the epithelium (left), lamina propria (middle) and submucosa (right) of the trachea 18 hr after saline or OVA challenge. DC density is given as the number of positive cells/mm^2 in each tracheal compartment. n = 7 or 8 for OVA-treated animals, n = 4 for saline-treated animals. * P < .05 compared with saline-treated animals; # P < .05, OVA *vs.* OVA + FP.

saline-treated or saline plus FP-treated animals (fig. 2). Surprisingly, FP administration did appear to increase DC number in the lamina propria of saline-treated animals (fig. 2). An explanation for this increase is not known. In the bronchi, FP treatment inhibited by 97% and 105% the OVA-induced increase in DCs in the lamina propria and adventitia, respectively (fig. 3), to levels that were not different from the DC prevalence in saline-treated or saline plus FP-treated animals (fig. 3).

In addition to these anti-inflammatory effects, FP abolished the development of airway hyperreactivity after OVA challenge (fig. 4). Comparison of reactivity to MCh before OVA sensitization and 18 hr after challenge revealed that the MCh PC_{200} (fig. 4) was not decreased in animals treated with FP. There was a trend (P < .07) toward hyporeactivity to MCh in FP-treated animals. However, these changes were not statistically significant.

Effects of PI on obstruction, inflammation and airway hyperreactivity in response to OVA challenge. The early elevation in SRaw after OVA challenge was not affected by PI treatment (Lawrence *et al.*, 1997). The administration of PI aerosol before OVA challenge produced anti-inflammatory effects similar to those seen after FP administration. OVA-induced eosinophil influx was reduced significantly by 70% in the tracheal epithelium after PI administration to a level that remained significantly greater than the value observed in the saline-treated animals (fig. 5); that is, PI did not completely inhibit the OVA-induced eosinophil response.

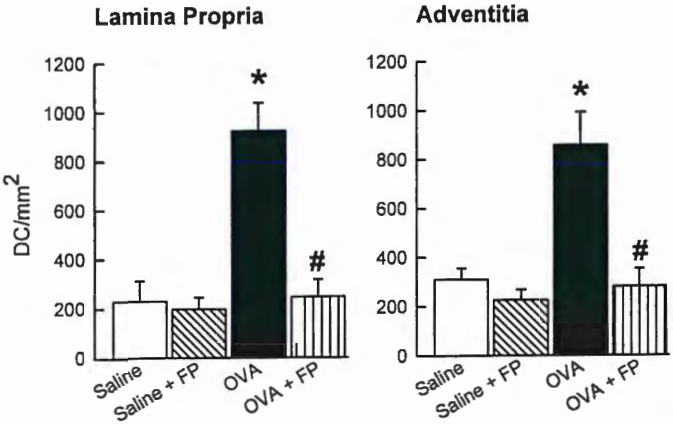

Fig. 3. Effect of FP treatment on DC prevalence in the lamina propria (left) and adventitia (right) of the bronchi 18 hr after saline or OVA challenge. DC density is given as the number of positive cells/mm^2 for each tracheal compartment. n = 7 or 8 for OVA-treated animals, n = 4 for saline-treated animals. * P < .05 compared with saline-treated animals; # P < .05, OVA *vs.* OVA + FP.

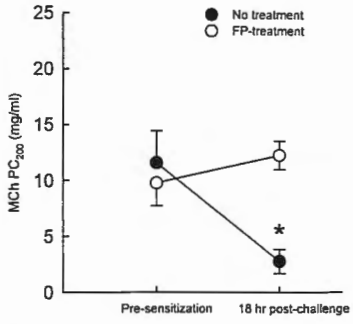

Fig. 4. Effect of FP treatment on the development of airway hyperreactivity after sensitization and challenge with OVA. MCh dose-response curves were generated before sensitization with OVA and 18 hr after OVA challenge (18 hr post-challenge) in untreated (●) and FP-treated (○) guinea pigs. There was a significant decrease in the [MCh] PC_{200} 18 hr after challenge in untreated but not FP-treated animals. There was no significant difference in PC_{200} values before OVA challenge. A decrease in PC_{200} indicates an increase in airway reactivity. n = 8 for each treatment group. * P < .05 compared with presensitization values.

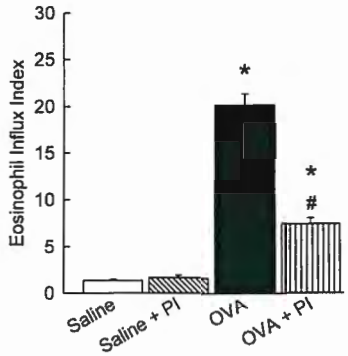

Fig. 5. Effect of PI treatment on eosinophil prevalence in tracheal epithelium 18 hr after saline or OVA challenge. The Eosinophil Influx Index represents the percentage of the epithelial area that was DAB positive. n = 7 or 8 for OVA-treated animals, n = 4 for saline-treated animals. * P < .05 compared with saline-treated animals; # P < .05, OVA *vs.* OVA + PI.

Tissue macrophage density was unaffected by PI administration in OVA-treated animals (data not shown).

In the trachea, PI inhibited by 100% and 48% the OVA-induced increase in DCs in the epithelium and lamina propria, respectively (fig. 6). However, the number of DCs in the lamina propria after PI treatment remained significantly elevated compared with that observed in saline-treated animals (fig. 6). PI had no effect on the DC response in the submucosal region of the trachea (fig. 6). In the bronchi (fig. 7), PI significantly inhibited the OVA-induced increase in DC in the lamina propria by 75%. No significant reduction in DC

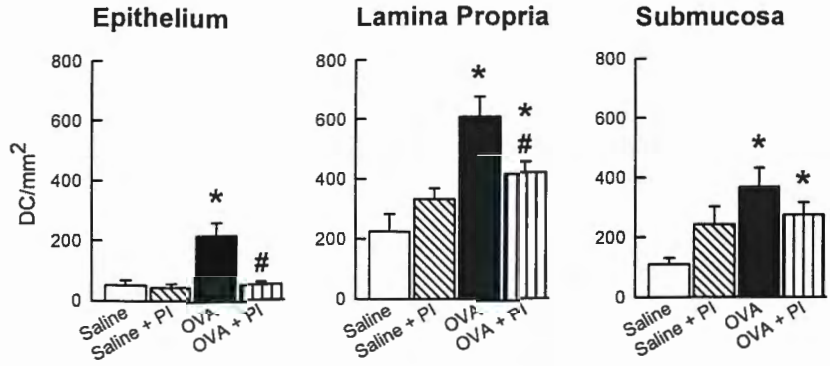

Fig. 6. Effect of PI treatment on DC prevalence in the epithelium (left), lamina propria (middle) and submucosa (right) of the trachea 18 hr after saline or OVA challenge. DC density is given as the number of positive cells/mm^2 in each tracheal compartment. n = 7 or 8 for OVA-treated animals, n = 4 for saline-treated animals. * P < .05 compared with saline-treated animals; # P < .05, OVA *vs.* OVA + PI.

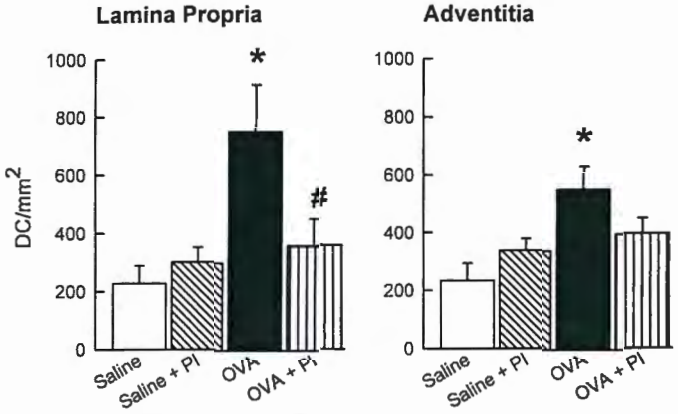

Fig. 7. Effect of PI treatment on DC prevalence in the lamina propria (left) and adventitia (right) of the bronchi 18 hr after saline or OVA challenge. DC density is given as the number of positive cells/mm^2 for each tracheal compartment. n = 7 or 8 for OVA-treated animals, n = 4 for saline-treated animals. * P < .05 compared with saline-treated animals; # P < .05, OVA *vs.* OVA + PI.

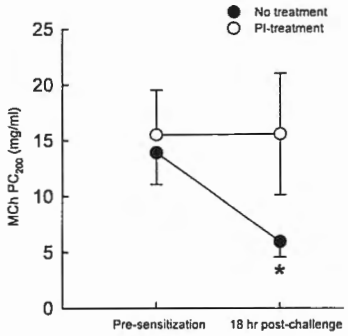

Fig. 8. Effect of PI treatment on the development of airway hyperreactivity after sensitization and challenge with OVA. MCh dose-response curves were generated before sensitization with OVA and 18 hr after OVA challenge (18 hr post-challenge) in untreated (●) and PI-treated (○) guinea pigs. There was a significant decrease in the [MCh] PC_{200} 18 hr after challenge in untreated but not PI-treated animals. There was no significant difference in PC_{200} values before OVA challenge. A decrease in PC_{200} indicates an increase in airway reactivity. n = 8 for each treatment group. * P < .05 compared with presensitization values.

prevalence in the adventitial region was observed after PI treatment (fig. 7).

PI treatment abolished the increase in airway reactivity to MCh induced by OVA challenge (fig. 8).

Discussion

Inhaled glucocorticoids are effective in preventing the inflammation observed in asthmatics (Schleimer, 1993). Although these compounds have been shown to act on many of the cell types involved in antigen-induced lung inflammation (Schleimer, 1990), their effect on DC in the airways has not been investigated in the guinea pig. FP given by inhalation before OVA challenge significantly reduced the elevation in the prevalence of DC that would ordinarily occur in all tracheal and bronchial compartments. In conjunction with the inhibited DC response was a decrease in eosinophil influx into the tracheal epithelium. Last, the development of airway hyperreactivity to MCh was prevented.

Previous studies of glucocorticoid effects on allergen-induced responses in the guinea pig have focused primarily on alterations of inflammatory cell influx into the airways with little attention being paid to concomitant changes in pulmonary function and airway responsiveness. Oral administration of methylprednisolone at 2 and 24 hr before OVA challenge significantly reduced pulmonary eosinophilia (Chand *et al.*, 1990). Likewise, the administration of dexamethasone (Tarayre *et al.*, 1991) and betamethasone (Gulbenkian *et al.*, 1990) to OVA-treated guinea pigs was shown to decrease eosinophil influx into the airways. The administration of dexamethasone before OVA challenge decreased both eosinophil and $CD4^+$ T lymphocyte infiltration of the bronchial wall (Lapa e Silva *et al.*, 1995).

The only previous report of the effects of FP on pulmonary DC was obtained using rats (Nelson *et al.*, 1995). FP pretreatment for 7 days in a rat model of respiratory viral infection decreased DC number by 50%. This finding, as well as the results of the present study, reveals that steroids can modify the pulmonary DC response and supports the idea that DC are an important component in the initiation of pulmonary inflammation, whether virus or allergen induced.

PI is a compound that is not currently used in the management of asthma but has been shown in animal models to possess anti-inflammatory properties. The drug inhibits B lymphocyte responses (Ferrante and Secker, 1985), reduces antigen presentation by Langerhans cells (Blaylock *et al.*, 1991) and inhibits the production of numerous cytokines such as tumor necrosis factor-α, interleukin-1 and interleukin-6 (Corsini *et al.*, 1992; Rosenthal *et al.*, 1991, 1992). The administration of PI reduced OVA challenge-induced eosinophil influx into the tracheal epithelium, prevented the development of airway hyperreactivity to MCh and reduced DC prevalence in the tracheal epithelium and tracheal and bronchial lamina propria. However, in contrast to FP, PI treatment did not affect DC number in either the tracheal submucosa or bronchial adventitia. Why DC prevalence

remained unchanged in the deeper regions of the tracheal and bronchial walls remains to be seen. It could be related to the limitations on the uptake and availability of PI into the walls of the airways. Studies in both rats and mice have shown that aerosolized PI produces high, sustained lung tissue levels (Debs *et al.*, 1987), but this could reflect absorption through the walls of smaller airways or alveoli. Therefore, it is possible that increasing the dose or number of treatments would allow greater deposition of the drug. On the other hand, the anti-inflammatory effects of FP and PI may not be the same at any dose of PI.

In conclusion, we developed an animal model of asthma in which increases in airway DC, airway inflammation and airway hyperreactivity are concurrently observed. The administration of FP, an anti-inflammatory drug, and PI, an antiprotozoal compound with known anti-inflammatory effects, inhibited the OVA-induced increase in DC prevalence in the airways while reducing the levels of other inflammatory cells and preventing the development of airway hyperreactivity. These results suggest a possible correlation between the events leading to the increase in pulmonary DC prevalence and the development of lung inflammation and airway hyperreactivity. Our study suggests that DCs, which are at the top of the inflammation cascade initiated by antigen, may be targets for the effects of currently available and future drugs for the treatment of asthma. Inhibition of DCs may prevent the development of airway hyperresponsiveness.

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