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Inhibitory effect of cyclosporin A on eosinophil infiltration in the guinea-pig lung induced by antigen, platelet-activating factor and leukotriene B_4

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ABSTRACT: The effect of the immunosuppressive compound, cyclosporin A, and the corticosteroid, betamethasone, was investigated on eosinophil accumulation in guinea-pig lung tissue induced by antigen, platelet-activating factor (PAF) and leukotriene B_4 (LTB_4).

The accumulation of eosinophils in the peribronchial area was evaluated on histological preparations. The lung sections were stained with Luna's reagent specific for eosinophil granule content.

Oral treatment of the guinea-pigs with cyclosporin, 10 $mg \cdot kg^{-1}$ three times a day for two days, and 10 $mg \cdot kg^{-1}$ 1 h before antigen challenge, significantly reduced the accumulation of eosinophils observed at 4 and 24 h, in the peribronchial area of sensitized guinea-pig lung. Betamethasone (3 $mg \cdot kg^{-1}$), administered orally 24 h and 1 h before antigen challenge elicited a moderate but significant reduction of antigen-induced eosinophil accumulation. Pretreatment of the guinea-pigs with cyclosporin or betamethasone elicited a marked inhibition of the accumulation of eosinophils in the peribronchial area induced by aerosolized PAF (100 $\mu g \cdot ml^{-1}$) or LTB_4 (5 $\mu g \cdot ml^{-1}$).

Since cyclosporin and betamethasone significantly inhibit the antigen-induced eosinophil accumulation, these results suggest that antigen-induced lung eosinophilia is dependent of T-lymphocytes. However, cyclosporin and betamethasone may also reduce the chemotactic activity of PAF and LTB_4 on guinea-pig eosinophils.

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Airway eosinophilia is a major characteristic of allergic diseases, particularly of the bronchopulmonary system, as in asthma [1]. Recent evidence has suggested that the T-lymphocyte may play an important role in atopic allergic inflammation, such as the recruitment of eosinophils in lung tissue. The presence of activated T-cells in bronchial biopsies of asthmatics [2] has led to the belief that these cells may be responsible for both initiation and maintenance of chronic allergic inflammation. Furthermore, there is growing evidence that a close relationship may exist between CD4+ T-cells and eosinophils in allergic inflammation [3–5]. Moreover, activated T-cells are able to release cytokines, such as interleukin-5 (IL-5), which are involved in the differentiation, recruitment and activation of eosinophils [5–9].

Several animal models of allergen-induced bronchial hyperresponsiveness and accumulation of eosinophils in lung tissue, have been described. We recently developed a model in which both initial sensitization and subsequent exposure to antigen are performed by aerosol [10]. In this study, an enhanced bronchopulmonary response to aerosol administration of acetylcholine, 5-hydroxytryptamine and substance P was observed 4–48 h after ovalbumin exposure of sensitized animals. In addition, histological examination of lung specimens obtained following challenge demonstrated eosinophil infiltration in the peribronchial regions and bronchial walls, as well as within the epithelium [10, 11].

In order to characterize the role of T-cells in antigen-induced lung eosinophilia, we investigated the effect of the T-cell-selective immunosuppressive product cyclosporin A. Cyclosporin A is a cyclic peptide of fungal origin, which is used to prevent rejection in organ transplantation and has a selective inhibitory effect on T-lymphocytes by inhibiting lymphokine production and release, for example interleukin-2 (IL-2) [12, 13]. The effect of cyclosporin was also compared to that of the glucocorticoid, betamethasone, on lung eosinophilia induced by antigen, platelet-activating factor (PAF) and leukotrine B_4 (LTB_4).

Materials and methods

Sensitization procedure and antigen exposure

For sensitization, male Hartley guinea-pigs (350–400 g) were placed twice, at a 48 h interval, in a plexiglass chamber (30×50×30 cm) and exposed to aerosols of a 0.9% NaCl solution in water (saline solution) containing 2 $mg \cdot ml^{-1}$ ovalbumin (OA) for 30 min. The aerosol was generated by a Devilbiss ultrasonic nebulizer (ULTRA-NEB 99, Sommerset, Pennsylvania, USA).

Fifteen to 20 days after the initial sensitization procedure, the guinea-pigs were challenged in the plexiglass chamber, by exposure to five successive solutions of OA, respectively, 10 $\mu g \cdot ml^{-1}$, 100 $\mu g \cdot ml^{-1}$, 1 $mg \cdot ml^{-1}$, 5 $mg \cdot ml^{-1}$ and 10 $mg \cdot ml^{-1}$ for 15 min each. The aerosol was generated with the ULTRA-NEB 99 DeVilbiss nebulizer, as described above. These increasing concentrations were used to avoid fatal anaphylactic reactions [10]. As a control group, sensitized guinea-pigs were exposed to an aerosol of the saline solution for 30 min.

Platelet-activating factor and LTB_4 exposures

Nonsensitized guinea-pigs were exposed in the plexiglass chamber for 30 min to different saline solution containing 0.1% bovine serum albumin (BSA) with PAF (maximum activity observed with 100 $\mu g \cdot ml^{-1}$), or LTB_4 (5 $\mu g \cdot ml^{-1}$). The aerosol was generated by a Devilbiss ultrasonic nebulizer (ULTRA-NEB 99), as described above. No apparent sign of respiratory failure was observed in the guinea-pigs. Control guinea-pigs were exposed to aerosols of the saline solution with BSA (control for PAF) or without BSA (control for LTB_4).

Treatment of guinea-pigs

Cyclosporin A was administered orally, 10 $mg \cdot kg^{-1}$ three times a day for two days, and 10 $mg \cdot kg^{-1}$ 1 h before either OA challenge or PAF or LTB_4 exposure. Betamethasone (3 $mg \cdot kg^{-1}$) was administered orally, either 24h and 1 h, or only 1 h, before OA challenge or PAF or LTB_4 exposure.

Histological techniques

Lungs from anaesthetized guinea-pigs were removed 4 h or 24 h after ovalbumin, PAF, LTB_4 or saline exposure. The lungs were washed, by injection through the pulmonary artery with 10 ml of saline solution. The lung tissue was fixed in 10% buffered formalin, embedded in paraffin, and 5 µm slices were cut and stained with Luna's reagent specific for the eosinophil granule content [14]. Histological preparations were observed with a Leitz Aristoplan microscope using a 40× magnification. For each guinea-pig, three slices were prepared at three different levels of the large left lobe, in order to observe the eosinophil infiltration in airways of various calibre. Under these conditions, the mean diameter of the airways examined was 150 µm. The number of eosinophils was evaluated in the peribronchial area, which included all the bronchial wall, *i.e.* the epithelium, the lamina propria and the submucosa. All the bronchi/bronchioles (n=35–40) of each section were selected. For each preparation, eosinophils were counted blind using a computerized light analyser (Leitz, Rueil-Malmaison, France and IMSTAR, Paris, France).

Materials

Ovalbumin (OA, chicken egg, grade V) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Cyclosporin A (Sandimmun, Sandoz, Basel, Switzerland) was diluted in olive oil, at the final concentration of 10 $mg \cdot ml^{-1}$. PAF (C_{16}) (Novabiochem, Cléry en Vexin, France) was prepared in saline solution (NaCl, 0.9%) containing 0.1% BSA. The others drugs were urethane (ethylcarbamate, Prolabo, Paris, France); pancuronium bromide (Pavulon, Organon, Fresnes, France); and betamethasone (Célestène, Shering Plough, Levallois Perret, France). Leukotriene B_4 (LTB_4) was synthesized at the Institut Henri Beaufour, dissolved in distilled water and further diluted in saline solution. Stock solutions of each compound were prepared daily. Specific pathogen-free male Hartley guinea-pigs (350–400 g) were obtained from Charles-River (St Aubin les Elbeuf, France). They were bred and housed in our standard animal care facilities. All guinea-pigs were fed standard pellets (UAR, Villemoisson-sur-Orge, France) and given water *ad libitum*. For each experimental group, at least five animals were used.

Expression of the results and statistical analysis

Results are expressed as mean±standard error of the mean (SEM). Statistical differences between eosinophil number in lung tissue have been compared by the non-parametric Mann Whitney U-test.

Results

Effect of cyclosporin on antigen-induced eosinophil accumulation

Four and 24 h after saline exposure of sensitized guinea-pigs, no significant eosinophil accumulation was observed, as detected by the red coloration after Luna staining, although eosinophils were present in small numbers in the peribronchial area. By contrast, 4 h after OA exposure of sensitized animals, significant accumulation of eosinophils was recorded (fig. 1). Pretreatment of the guinea-pigs with cyclosporin (10 $mg \cdot kg^{-1}$) significantly ($p<0.001$) reduced the antigen-induced eosinophil accumulation in the peribronchial area observed at 4 h (fig. 1). When the lungs were removed 24 h after OA exposure, a significant ($p<0.001$) inhibition was also observed after pretreatment of the guinea-pig with cyclosporin (fig. 1).

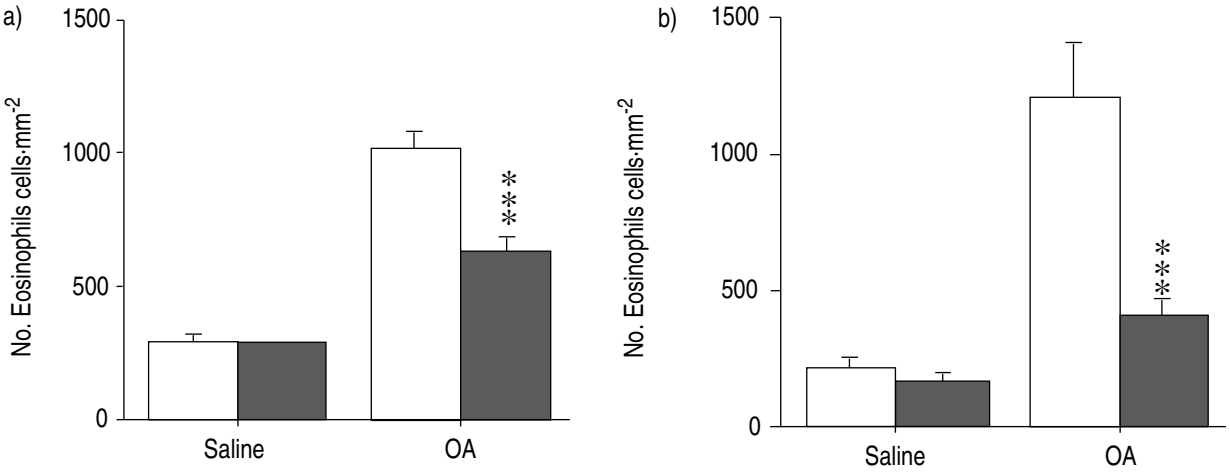

Fig. 1. – Effect of cyclosporin A on the eosinophil numbers in the peribronchial area of the pulmonary tissue, 4 h (a) or 24 h (b) after aerosol administration of a saline solution with or without ovalbumin (OA). Cyclosporin was administered orally, 10 mg·kg^{-1} three times a day for two days, and 10 mg·kg^{-1} 1 h before ovalbumin challenge. Comparison with the respective control: ***: p<0.001 (n= 5–6). ☐ : control; ■ :

Effect of betamethasone on antigen-induced eosinophil accumulation

Betamethasone administered orally at the dose of 3 mg·kg^{-1}, 1 h before antigen exposure did not reduce the eosinophil accumulation observed at 24 h (fig. 2). By contrast, when betamethasone was administered twice, 24 h and 1 h before antigen challenge, a moderate but significant (p<0.05) reduction in the accumulation of eosinophils was noted (fig. 2).

Effect of cyclosporin on PAF- or LTB_4-induced eosinophil accumulation

When administered by aerosol for 30 min, PAF induced a dose-dependent increase in eosinophil number in the peribronchial area, 24 h later. Significant increase was only recorded with PAF at or above 50 μg·m^{-1}; the maximum eosinophil number was noted with 100 μg·ml^{-1} PAF. Pretreatment of the guinea-pigs with cyclosporin (10 mg·kg^{-1}) elicited a marked inhibition of the PAF-induced accumulation in the peribronchial area (fig. 3).

LTB_4 (5 μg·ml^{-1}) administered by aerosol for 30 min induced a potent eosinophil accumulation, 24 h later. The magnitude of the eosinophil accumulation induced by LTB_4, was the same as observed with the high concentration of PAF (100 μg·ml^{-1}) (fig. 3). Pretreatment of the guinea-pigs with cyclosporin (10 mg·kg^{-1}) also induced a significant reduction of the increase in eosinophil number in lung tissue (fig. 3).

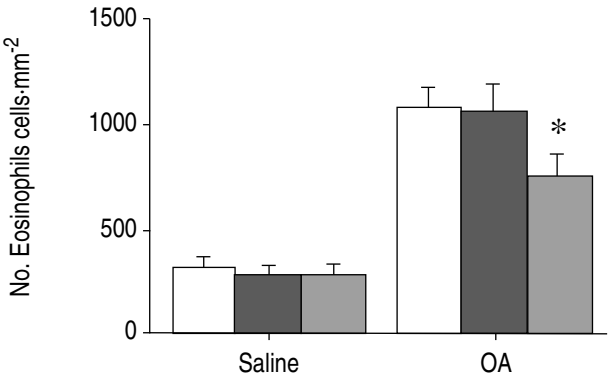

Fig. 2. – Effect of betamethasone on the increase in eosinophil numbers in the peribronchial area of the pulmonary tissue, 24 h after aerosol administration of a saline solution with or without ovalbumin (OA). Betamethasone was administered orally (3 mg·kg^{-1}), either 24 and 1 h (treatment B) or only 1 h (treatment A), before ovalbumin challenge. Comparison with the respective control: *: p<0.05 (n=5–6). ☐ : control; ■ : treatment A, ☐ : treatment B.

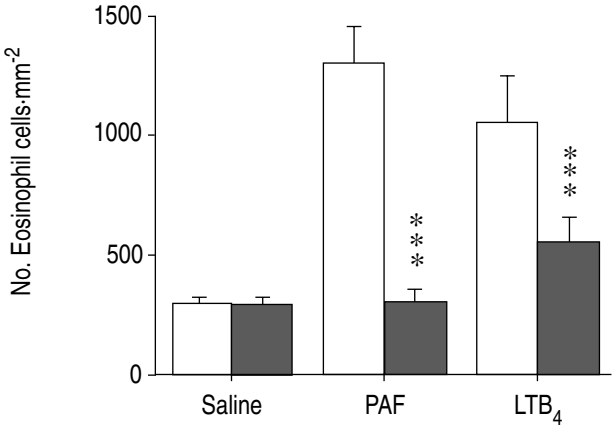

Fig. 3. – Effect of cyclosporin A on the increase in eosinophil numbers in the peribronchial area of the pulmonary tissue, 24 h after aerosol administration of a saline solution (with or without BSA) or PAF (with BSA) (100 μg·ml^{-1}) or LTB_4 (5 μg·ml^{-1}) for 30 min. Cyclosporin A was administered orally, 10 mg·kg^{-1} three times a day for two days and 10 mg·kg^{-1} 1 h before PAF or LTB_4 exposure. Comparison with the respective control: ***: p<0.00l (n=5–6). ☐ : control; ■ : cyclosporin A. BSA: bovine serum albumen; PAF: platelet-activating factor; LTB_4: leukotriene B_4.

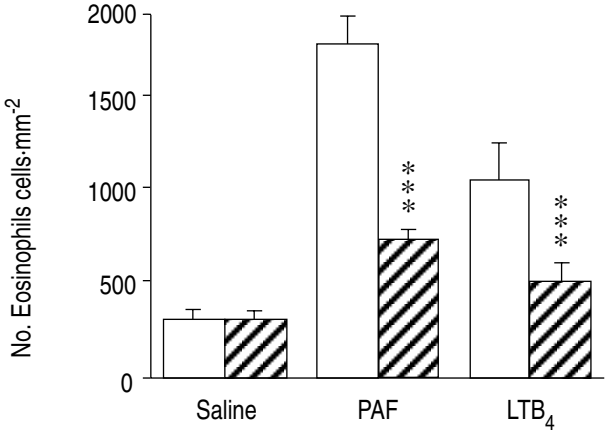

Fig. 4. – Effect of betamethasone administered orally (3 mg·kg^{-1}) on the increase in eosinophil numbers in the peribronchial area of the pulmonary tissue, 24 h after aerosol administration of a saline solution with or without BSA or PAF (100 mg·ml^{-1}) or LTB_4 (5 μg·ml^{-1}) for 30 min. Betamethasone was administered orally (3 mg·kg^{-1}), 24 and 1 h, before PAF or LTB_4 exposure. Comparison with the respective control: ***: $p<0.001$ (n=5–6). □ : control; ▨ : betamethasone. For abbreviations see legend to figure 3.

Effect of betamethasone on PAF- or LTB_4-induced eosinophil accumulation

Betamethasone administered orally at the dose of 3 mg·kg^{-1}, 24 and 1 h before PAF or LTB_4 exposures, significantly reduced the eosinophil accumulation in the peribronchial area at 24 h (fig. 4).

Discussion

The present data show that cyclosporin and betamethasone reduce the eosinophil accumulation induced by antigen, PAF and LTB_4 in guinea-pig lung tissue. With regard to the immunosuppressive activity of cyclosporin and betamethasone on T-lymphocytes, these results support the hypothesis that allergen-induced eosinophilia in the airways is partially dependent on the activation of T-lymphocytes. It has previously been reported that cyclosporin inhibits antigen-induced eosinophil accumulation in guinea-pig and Brown-Norway rat lungs [15, 16]. However, in these experiments, in contrast to our studies, the number of eosinophils was evaluated in the bronchoalveolar lavage fluid. Nevertheless, marked difference have been demonstrated between the alveolar space (as reflected by the cell composition of the bronchoalveolar lavage fluids) and the lung tissue (as assessed by histological techniques) [l0]. Norris *et al*. [15] showed a significant inhibition of lung eosinophilia assessed by lavage when cyclosporin was administered at the time of sensitization, but not when administered for 3 days before lavage. Since the sensitization procedure is dependent on T-cell cooperation to regulate antibody formation, which triggers the challenge response, it is not surprising that cyclosporin was effective when administered during the sensitization period.

It is probable that the immunosuppressive effect of cyclosporin A is mediated through the suppression of T-lymphocyte activation [17], particularly of the CD4+ helper/inducer subset, through inhibition of T-cell growth factor (IL-2) [18, 19]. However, it also appears that cyclosporin A inhibits the production of other lymphocyte-derived cytokines *in vitro* [20, 21]. Interestingly, the release of interleukin-5 (IL-5), which is a growth factor and a chemotactic factor for eosinophils, is also inhibited by cyclosporin A [22]. Moreover, challenge of sensitized animals with aerosolized OA was followed by an increase in mucosal T-cells numbers in guinea-pigs [4, 23] and in Brown-Norway rats with a recruitment of CD3+, CD4+ and CD25+ T-lymphocytes [24]. Therefore, we could expect other interleukins, such as IL-5, to be released from these infiltrating cells, leading to lung eosinophilia. Interestingly, the concept that T-lymphocytes may be involved in allergen-induced bronchial hyperresponsiveness is strengthened by the recent observation that IL-2 induces bronchial hyperresponsiveness to methacholine in rats and causes tissue infiltration of lymphocytes and eosinophils [25].

As with cyclosporin A, betamethasone also significantly reduced eosinophil infiltration after antigen challenge. Glucocorticoid therapy is one of the most effective anti-inflammatory treatments available in asthma [26], with potent inhibitory effects against bronchopulmonary alterations following allergen challenge in atopic asthmatic patients [27, 28]. The precise mechanisms of action of glucocorticoids is at present not clear. However, it is likely that part of the therapeutic action of glucocorticoids in asthma derives from an inhibitory activity at various levels of the inflammatory process and on several different cell types, including lymphocytes [17], with inhibition of the release of mediators, such as cytokines [25]. Moreover, they inhibit the activation of macrophages and monocytes [29], and of eosinophils [30]. Corticosteroids, therefore, possess a wide range of properties which may explain the effects observed.

Pretreatment of the guinea-pigs with cyclosporin or betamethasone elicited a marked inhibition of aerosolized PAF or LTB_4-induced accumulation of eosinophils in the peribronchial area. This indicates that these two drugs may interfere directly with the recruitment of eosinophils. Indeed, PAF and LTB_4 induced direct migration of eosinophils *in vitro* [31–33]. A direct effect of steroids on eosinophils and their function has been proposed [34, 35]. However, to our knowledge, no experimental evidence has been reported that cyclosporin reduces the chemotactic activity of PAF and LTB_4 *in vitro*. An indirect inhibitory activity of cyclosporin and betamethasone through the reduction of T-lymphocyte activation and recruitment and chemotactic factor release is not excluded. Finally, experiments are in progress to determine whether PAF and LTB_4 are able to induce T-lymphocyte recruitment in the lung, as is the case for antigen challenge in sensitized rats.

Since cyclosporin and betamethasone significantly inhibit antigen-induced eosinophil accumulation, these

results suggest that antigen-induced lung eosinophilia is dependent on T-lymphocytes. However, further studies are needed to determine whether cyclosporin and betamethasone possess the property of reducing the *in vivo* chemotactic activity of PAF and LTB_4 on eosinophils in the guinea-pig. This would ultimately provide further evidence of a possible therapeutic role for cyclosporin in pulmonary inflammation and asthma, as has been proposed previously [36, 37].

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