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Progesterone increases airway eosinophilia and hyper-responsiveness in a murine model of allergic asthma

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Summary

Background Sex hormones might affect the severity and evolution of bronchial asthma. From existing literature, there exists, however, no convincing evidence for either exacerbation or improvement of allergic symptoms by progesterone.

Objective This study was aimed to explore the effect of exogenously administered progesterone in a mouse model of allergic asthma.

Methods BALB/c mice were sensitized to ovalbumin (OVA) by intraperitoneal injections with OVA followed by chronic inhalation of nebulized OVA or physiologic saline (Sal). Medroxyprogesterone acetate or placebo was instilled daily into the oesophagus before and during the inhalatory OVA challenge phase.

Results Progesterone worsened allergic airway inflammation in OVA-challenged mice, as evidenced by enhanced bronchial responsiveness to inhaled metacholine and increased bronchial eosinophilia. Elevated airway eosinophilia corresponded with higher bronchial and systemic IL-5 levels in the progesterone group. The ratio of IL-4/IFN-γ levels in bronchoalveolar lavage fluid and numbers of eosinophil colony-forming units in the bone marrow were also elevated in the latter group. Progesterone, however, did not influence allergen-specific IgE production, nor did it affect bronchial responses in Sal-challenged mice.

Conclusion Our data show that exogenously administered progesterone aggravates the phenotype of eosinophilic airway inflammation in mice by enhancing systemic IL-5 production. Progesterone also increases bronchial hyper-reactivity.

Keywords airway responsiveness, allergic asthma, eosinophils, mouse model, progesterone
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Introduction

Epidemiologic data illustrate that female sex hormones are likely to be involved in the pathology of allergic asthma. Severe asthmatic patients have a higher female/male ratio (3 : 1) [1] and women have a higher risk of hospital admission for asthma than men [2]. Moreover, female asthmatic patients show a significant increase in airway reactivity during the luteal phase [3] and one-third of asthmatic women report an increase in asthma symptoms during the premenstrual period [4]. Consistently, almost half of all female admissions for acute asthma occur during the perimenstrual phase [5]. In this regard, female sex hormones have been pointed out to be potentially involved in worsening allergic symptoms. Indeed, 80% of female asthmatics have at least one of the following hormones, i.e. estradiol, progesterone and cortisol outside the normal range [6], suggestive of an association between asthma and alteration in the production and metabolism of steroid hormones.

At present, convincing evidence for either beneficial or deleterious effects of female sex hormones in general, and progesterone in particular, on allergic asthma is lacking. *In vitro* studies showed that progesterone skews the differentiation of naive T helper (Th) lymphocytes towards the Th2 lineage [7] and induces the production of IL-4 in Th1-like clones [8]. This effect is manifested via a progesterone-induced factor that blocks Th1 responses [9]. Consistent with these *in vitro* data, a predominant Th2 cytokine profile has been demonstrated in stimulated T cells of healthy women [10]. The so-called Th2 cytokines IL-4, IL-5 and IL-13 each contribute substantially to the phenotype of allergic airway inflammation [11], with bronchial eosinophilia and hyper-responsiveness as major hallmarks. Regarding the Th2-potentiating effect of progesterone *in vitro*, one would expect that progesterone aggravates the manifestation of allergic asthma. Indeed, oral contraceptives have been reported to have some asthma-promoting potential [12]. However, other studies failed to confirm this observation [2, 13–15]. Remarkably, even beneficial effects of progesterone have

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been observed by others [16–18]. While interpreting results of studies on progesterone and asthma, it should be noted that differences in patient selection, in doses of sex hormones administered and in the modulation of endogenous progesterone levels may all determine the outcome of the study. In addition, the effects of exogenously administered progesterone on allergic asthma in clinical trials could often not be dissociated from the effects of the co-administered estrogens [13, 14].

Besides modulation of Th responses, sex hormones also influence smooth muscle contractions and may thus influence the clinical presentation of airway hyper-responsiveness (AHR) in asthmatic patients. At present, there is no consensus on the precise role of progesterone on airway smooth muscle contractile properties. Progesterone enhances airway responsiveness to hypoxia and hypercapnia in healthy men [19] and reverses the oestrogen-induced increase in the number of β-adrenergic receptors in rabbit pulmonary tissue [20]. On the other hand, progesterone dampens airway responsiveness through up-regulation of β_2-adrenoceptors in healthy women [21], potentiates isoprenaline-induced bronchial relaxations in pigs [22] and prevents histamine-induced contractions in the guinea-pig trachea [23]. These apparently contradictory results may be related to different experimental settings and doses of progesterone tested. It is also noteworthy to mention that one cannot extrapolate data obtained in non-inflamed to inflamed bronchi. For example, exogenous progesterone given during the follicular phase to healthy women produced significant up-regulation of β2-adrenoceptor expression [21], whereas it down-regulated and desensitized β2-adrenoceptors in female asthmatics [24].

Taken together, progesterone should be considered among the determinants of asthma severity. At present, it remains unclear whether asthma exacerbation during the premenstrual period in a subgroup of female asthmatics is due to objectively measurable intensification of airway disease or to increased perception of symptoms caused by the particular physiological state occurring shortly before menstruation. Here, we investigate the immunomodulatory capacity of progesterone on the manifestation of allergic asthma in a chronic mouse model of allergic asthma [25]. In order to avoid endogenous production of significant amounts of female sex hormones and to bypass cyclic changes in female hormone levels, male mice were chosen for this study.

Methods

Murine model of allergic asthma and progesterone treatment

BALB/c mice (males, 6 weeks of age) were obtained from Harlan (Horst, the Netherlands) and kept under conventional pathogenic-free conditions. Eosinophilic airway inflammation was induced with adjuvant-free OVA as previously described [25]. All mice were actively sensitized by seven i.p. injections of 10 μg OVA (grade V, Sigma, St Louis, MO, USA) in 0.5 mL of pyrogen-free saline (Sal) on alternate days (d) from d 1–13. From d 33–40, mice were exposed daily for 5 min to nebulized Sal (Sal group) or OVA (OVA group, 10 mg/mL, PARI TurboBOY, Starnberg, Germany). In addition, 50 μL of medroxyprogesterone acetate (500 mg/mL, Provera®, Pharmacia and Upjohn) or the same volume of vehiculum (glycerol, sodium carboxymethylcellulose, sorbitol and distilled water), as placebo (Plac) solution, was administered daily from d 26 till 40 into the oesophagus without anaesthesia by a non-traumatic catheter. Each group consisted of 12 mice. All mice were killed on d 41, i.e. 24 h after the eighth aerosol. For evaluation of cytokine production after allergen inhalation, the time-point of 6 h after allergen inhalation has been proven to be optimal [25]. Therefore, additional groups of similarly treated mice ($n = 12$) were killed at 6 h after the eighth aerosol. Experimental procedures were approved by the local Ethical Committee of Animal Experiments.

Measurement of airway responsiveness

On d 41, i.e. 24 h after the eighth aerosol, airway responsiveness was assessed by metacholine (Mch)-induced airflow obstruction in conscious mice placed in a whole body plethysmograph (Buxco®, EMKA Technologies, Paris, France) as described [26]. Airway responsiveness was evaluated by enhanced pause (P_{enh}), a calculated value that correlates with airway resistance [26]. $P_{enh} = (T_e/Tr - 1) \times (P_{ef}/P_{if})$ (T_e, expiration time; T_r, relaxation time; P_{ef}, peak expiratory flow; P_{if}, peak inspiratory flow). P_{enh} values were recorded for each mouse in rest and during 3 min after nebulizing physiologic Sal and incremental doses of Mch (5, 20 and 50 mg/mL) for 1 min as described previously [25]. The mean P_{enh} value of each mouse was calculated during one min at rest and at the time of maximal response after each dose of Mch.

Blood eosinophilia and serum levels of OVA-specific IgE

After measurement of AHR, mice were anaesthetized with urethane (i.p., 2.1 g/kg) and a retro-orbital bleed was performed. Blood smears were made and sera were frozen until analysis. The smears were stained according to the May–Grünwald–Giemsa (MGG) technique and the percentage of eosinophils in the white cell population was calculated. For ELISA, 96-well plates were first coated overnight with rat anti-mouse IgE (10 μg in 100 μL PBS, Pharmingen, San Diego, CA). Plates were blocked and incubated with 100 μL of diluted serum (1 : 5). After washing, the following substances were sequentially added, incubated and washed: OVA (1 μg/100 μL, Sigma), peroxidase-labelled rabbit anti-OVA IgG (240 ng/100 μL, Rockland, Gilbertsville, PA, USA) and buffer containing tetramethylbenzidin dihydrochloridhydrate (1 μL/100 μL, ACROS, New Jersey, NJ, USA) and H_2O_2 (1 μL/100 μL). The peroxidase reaction was stopped by adding H_2SO_4 and the optical density was measured at 450 nm. IgE levels of a reference pool of serum of highly OVA-sensitized mice were assigned a value of 1000 experimental units (EU) per mL.

Bronchoalveolar lavage (BAL) fluid

After exsanguination, lungs were lavaged five times through a tracheal polyethylene catheter (∅ 0.85 mm). The first lavage was performed with 1 mL of PBS supplemented with 5% BSA (Sigma) at 37 °C, centrifuged (1400 ***g***, 5 min) and the

supernatant was stored at –20 °C until measurement of cytokines and eosinophil peroxidase (EPO). The pellet was added to the subsequent four lavages performed with 1 mL of PBS. The BAL fluid pool was centrifuged (1400***g***, 5 min) and the cells were washed and resuspended in 200 μL of PBS. For cell counting, 10 μL of cell suspension were added to 90 μL of Türk's solution (Merck Diagnostica, Darmstadt, Germany) and the number of cells was calculated in a Bürker chamber. Differential cell counts were performed on MGG-stained cytospin preparations.

Histology

After performing BAL, lungs were obtained for histologic analysis. The maximal thickness of the inflammatory infiltrate around bronchioli and arterioli in the left and right lung were measured on haematoxylin and eosin (H&E) stained sections using an eyepiece graticule at a magnification of 25. The average of these 4 values was calculated for each mouse and expressed in 10^{-2} mM.

Bone marrow analyses

Bone marrow was collected from both femurs of 12 mice of each group. Smears were stained with the MGG stain and differential cell counts were performed blindly by one observer. Based on morphological criteria, cells were differentiated into blasts, myelocytic cells (promyelocytes, metamyelocytes and myelocytes), neutrophils, eosinophils, basophils, lymphocytes, monocytes and normoblasts. The presence of eosinophils was expressed in percentage of the total white cell count.

For progenitor quantification, 2×10^5 bone marrow cells were cultured with rmIL-5 (20 ng/mL, R&D Systems) in MethoCult M3230 (StemCell Technologies) as described [27]. After incubation for 18 d at 37 °C, the number of eosinophil CFUs was counted. The presence of eosinophil colonies was confirmed on MGG-stained cytospin preparations of the colonies.

Measurement of IL-5, IFN-γ and IL-4

IL-5 and IFN-γ levels in 1 : 2 diluted BAL fluid and serum were measured by sandwich ELISA using paired matched Abs according to the manufacturer's instructions (PharMingen). For IL-4 detection, an ELISA kit from Biosource (Fleurus, Belgium) was used. The lower limits of detection were 4.5, 2.0 and 1.2 pg/mL for IL-5, IL-4 and IFN-γ, respectively.

EPO assay

Undiluted BAL fluid (100 μL) and Tris-HCl buffer (50 μL) were added to 96-well plates. The samples were incubated for 10 min with 100 μL of substrate solution containing *o*-phenylenediamine (2 mg/mL, Sigma, St Louis, MO) and H_2O_2 (1.3 μL/mL) in Tris-HCl. A standard dilution (1/2) series containing horse-radish peroxidase (Merck, Darmstadt, Germany) starting at 200 ng/mL was made. The reaction was stopped by adding H_2SO_4 and the optical density was measured at 492 nm. The lower limit of detection was 12.5 ng/mL.

Data analyses

A Kruskal–Wallis test was performed to show differences in parameters between the four groups tested. When a parameter in one group was considered significantly different from the others, a Mann–Whitney test was performed to calculate the significance of the difference. With both methods, a difference was considered to be significant when $P < 0.05$. Data are expressed as means ± SEM.

Results

Effects of progesterone on pulmonary parameters of allergic airway inflammation

The sensitization protocol used here has been reported to induce cutaneous hypersensitivity reaction to OVA, production of OVA-specific IgE and to increase blood eosinophilia [25, 28]. Repeated challenges of sensitized mice with nebulized OVA (OVA mice) leads to eosinophilic airway inflammation and AHR [25, 28]. When progesterone (Pro) or medium (Plac) was administered during the challenge phase, OVA–Pro mice presented with enhanced AHR compared to OVA–Plac mice, as reflected by higher P_{enh} values ($P < 0.05$) after inhalation of Mch at 5 and 20 mg/mL on d 41 (Fig. 1a). Of note, intra-esophageal administration of progesterone did not alter AHR in Sal-challenged mice, as was illustrated by similar P_{enh} values in both groups of Sal mice (Sal–Plac and Sal–Pro, Fig. 1a).

Interestingly, progesterone markedly enhanced OVA-induced eosinophilic inflammation in the airways. Indeed, higher eosinophil counts were observed in the BAL fluid of OVA–Pro than OVA–Plac mice ($137.0 \pm 28.2 \times 10^4$/mL vs. $61.2 \pm 6.8 \times 10^4$/mL, $P < 0.05$, Fig. 1b). Consistent with the presence of more eosinophils in the bronchial lumen, OVA–Pro mice showed more EPO activity in the BAL fluid than OVA–Plac mice (95.3 ± 7.8 vs. 72.3 ± 11.3 pg/mL, $P < 0.05$, Fig. 1c).

Also in bronchial tissue, the inflammatory infiltrate was thicker in OVA–Pro than OVA–Plac mice ($156.2 \pm 11.3 \times 10^{-2}$ vs. $122.2 \pm 12.6 \times 10^{-2}$ mM, $P < 0.05$, Fig. 2b and d). As expected, both groups of Sal-challenged mice did not show any airway inflammation (Fig. 2a and c).

Effect of progesterone on cytokine levels in the BAL fluid

In order to study differences in cytokine profile by progesterone, additional groups of mice were killed at 6 h after the eighth challenge, because pilot studies showed that this time-point is optimal for measuring cytokine release in the BAL fluid and serum. In keeping with aggravation of allergic inflammation by progesterone, OVA–Pro mice had more IL-5 (48.2 ± 3.6 vs. 24.9 ± 8.2 pg/mL, $P < 0.05$) and IL-4 (195.2 ± 35.4 vs. 122.3 ± 12.2 pg/mL) in their BAL fluid, concomitant with less IFN-γ (2.7 ± 1.3 vs. 23.6 ± 2.1 pg/mL). In order to highlight the effect of progesterone on the cytokine balance in the BAL fluid, a ratio of the level of IL-4 over IFN-γ was made for each mouse and the means were calculated for each group. OVA–-Pro mice showed a higher ratio of IL-4/IFN-γ levels (227.0 ± 70.1 vs. 49.9 ± 7.5 pg/mL, $P < 0.05$, Fig. 1d) in the BAL fluid than OVA–Plac mice.

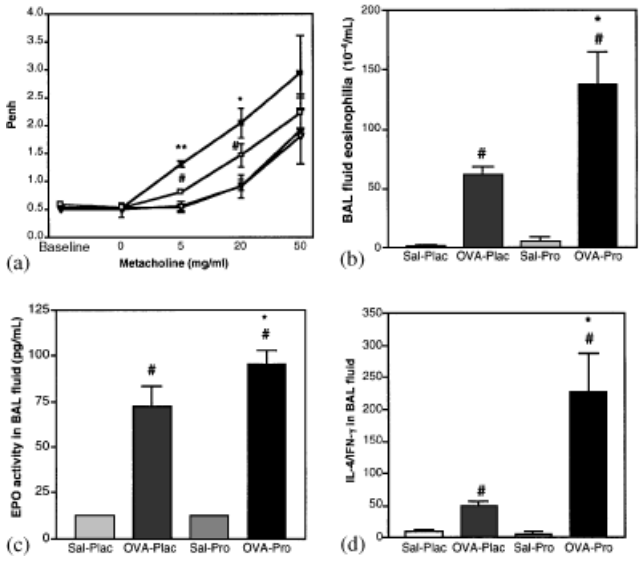

Fig. 1. Effects of progesterone on bronchial responsiveness and allergic inflammation. After sensitization to OVA, mice were exposed to nebulized saline (Sal, triangle) or OVA (square) and treated daily with either placebo (Plac, open symbol) or progesterone (Pro, filled symbol). On d 41, bronchial responsiveness (a) to incremental doses of inhaled Mch was evaluated by calculating Penh values using whole body plethysmography (Buxco, EMKA, France, $n = 12$ per group). Subsequently, BAL was performed and the number of eosinophils was determined (b, $n = 12$ per group). In addition, EPO activity was measured in the BAL fluid (c, $n = 12$ per group). At 6 h after the eighth challenge, levels of IL-4 and IFN-γ were measured in the BAL fluid using ELISA and the ratio of IL-4/IFN-γ levels was calculated (d, $n = 6$ per group). *, $P < 0.05$ vs. OVA-Plac, **, $P < 0.005$ vs. OVA–Plac, #, $P < 0.05$ vs. Sal mice.

There was no effect of progesterone on levels of IL-4, IL-5 and IFN-γ in the BAL fluid and in the serum of Sal-challenged mice (data not shown). At 24 h after the eighth challenge, there were no significant differences in cytokine levels in the BAL fluid or serum (data not shown).

Systemic effects of progesterone in allergic inflammation

During the whole experiment and at the time of killing (d 41), mice of all groups had similar weights (data not shown). Interestingly, serum levels of IL-5 were higher in OVA–Pro mice killed at 6 h after the eighth challenge, than in the OVA–Plac group (21.2 ± 4.2 vs. 9.4 ± 4.3 pg/mL, $P < 0.05$, Fig. 3a). In spite of IL-5 being primarily involved in stimulating eosinopoiesis and enhancing blood eosinophilia, similar percentages of eosinophils were found in peripheral blood of both OVA–Pro and OVA–Plac mice ($10.4 \pm 1.7\%$ and $9.1 \pm 1.7\%$, respectively). Progesterone did not influence IgE production, as demonstrated by similar levels of OVA-specific IgE in the serum of both Sal–Pro and Sal–Plac mice (884.4 ± 245.5 and 784.6 ± 211.2 EU/mL, respectively). Compared to Sal challenges, repeated OVA-challenges of sensitized mice slightly stimulated OVA-specific IgE production, in a non-statistically significant way ($P > 0.05$), in both progesterone and Plac-treated mice. Both groups of mice had equal titres of OVA-specific IgE (1227.0 ± 122.1 and 1238.0 ± 173.2 EU/mL, respectively).

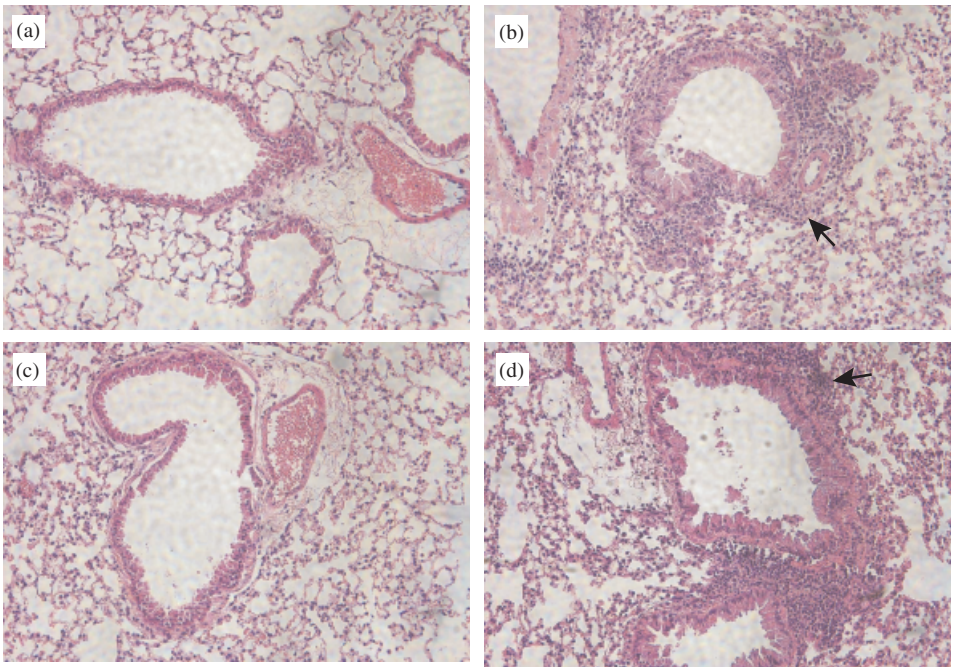

Fig. 2. Effects of progesterone on bronchial eosinophilic inflammation. Mice that had been sensitized and repeatedly challenged with nebulized saline (Sal, a and c) or OVA (d 33–40, b and d) were gavaged with placebo (Plac, a and b) or progesterone (Pro, c and d) during the challenge phase. Histologic analyses of murine airways were performed on d 41, i.e. after the 8th challenge with either Sal or OVA. OVA inhalations induced peribronchial inflammatory infiltrates (b and d), which was absent in Sal mice (a and c). Administration of progesterone during inhalatory OVA challenges aggravated the allergen-induced peribronchial eosinophilic inflammation (d), as reflected by thicker inflammatory infiltrates in OVA-Pro (d) than OVA–Plac mice (b). Microscopic images were made with the Axioplan microscope (Zeiss) at a magnification of 200 and are representative of the experimental group.

Effects of progesterone on bone marrow

OVA inhalations by sensitized mice significantly enhanced bone marrow eosinopoiesis, as suggested by the finding of higher percentages of eosinophils in OVA-challenged compared to Sal-challenged mice at 24 h after the eighth allergen challenge (18.4 ± 1.5 vs. $6.4 \pm 2.4\%$, $P < 0.05$). Treatment of both OVA and Sal-challenged mice with progesterone did not influence eosinophil percentages in the bone marrow (data not shown), nor did it alter the total bone marrow cell count (data not shown). However, eosinophil CFUs in OVA–Pro mice outnumbered those of OVA–Plac mice (16.7 ± 1.6 vs. 8.1 ± 0.6, $P < 0.005$, Fig. 3b). OVA–Plac mice had more eosinophil CFUs than Sal-Plac mice (8.1 ± 0.6 vs. 4.8 ± 1.4, $P < 0.05$, Fig. 3b), whereas equal numbers of eosinophil CFUs were found in both groups of Sal mice (Fig. 3b).

Discussion

In this manuscript, we show that exogenously administered progesterone aggravates the phenotype of allergic airway inflammation in BALB/c mice. This phenomenon is manifested by enhanced bronchial hyper-responsiveness and elevated airway eosinophilia. Moreover, aggravation of airway inflammation corresponded with elevated levels of IL-5 and with more eosinophil progenitor formation in the bone marrow. These data thus provide evidence for aggravation of allergic airway disease by exogenously administered progesterone.

Administration of progesterone during chronic allergen exposure also enhanced bronchial hyper-responsiveness to inhaled Mch in allergen-challenged mice. This effect of progesterone was restricted to allergen-induced airway inflammation, as progesterone and Plac-treated Sal-challenged mice showed similar airway responsiveness. Therefore, our data suggest that exogenous progesterone indirectly augments Mch-induced bronchoconstriction through aggravation of bronchial inflammation. Bronchial eosinophilia has been related to bronchial responsiveness in allergic airway inflammation [29], but a direct proof that eosinophils are responsible for hyper-reactivity is still lacking. However, other factors are undoubtedly involved in the pathophysiology of bronchial hyper-responsiveness [30, 31]. Compatible with this, we found a more prominent Th2 cytokine profile in the BAL fluid of progesterone-treated allergen-challenged mice. This observation in an *in vivo* model is consistent with the Th2-potentiating role of progesterone *in vitro*. Indeed, the presence of progesterone inhibits Th1 development and enhances IL-4 production *in vitro* [7]. Furthermore, Piccinni et al. [8] showed that progesterone skewed the cytokine production pattern of primed lymphocytes towards a more Th2-skewed cytokine profile. In line with *in vitro* data, higher levels of circulating IL-5 were found in progesterone-treated, allergen-challenged mice *in vivo*. Several cell types may be involved in the progesterone-mediated increase in IL-5 levels. *In vitro* studies have demonstrated that progesterone stimulates IL-5 secretion by activated memory T cells through up-regulation of IL-5 gene expression [32]. Also, mast cells and eosinophils are known to be IL-5-producing cells. However, the relevance of their progesterone receptor [33, 34] remains to be elucidated.

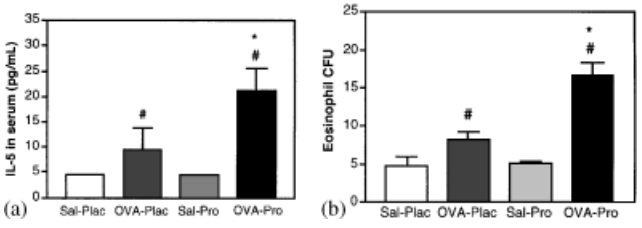

Fig. 3. Effects of progesterone administration on systemic levels of IL-5 and eosinopoiesis in the bone marrow. Mice that had been sensitized (d 1–13) and repeatedly challenged with nebulized OVA or saline (Sal) (d 33–40) were killed after the eighth allergen challenge. IL-5 levels were measured in serum using sandwich ELISA (A). After 18 d of culture of the bone marrow in the presence of IL-5, eosinophil CFUs were enumerated in culture plates (B, $n = 6$ for each group). #, $P < 0.05$ vs. similarly treated Sal-challenged mice and *, $P < 0.05$ vs. OVA–Plac.

Stimulation of IL-5 production by progesterone undoubtedly represents a key factor by which progesterone mediates aggravation of airway eosinophilia. As IL-5 has been demonstrated to be critical for eosinophil progenitor formation in the bone marrow [35], enhanced levels of IL-5 may explain the elevated number of eosinophil CFUs in the bone marrow of progesterone-treated mice with allergic airway inflammation. It is noteworthy to mention that bone marrow eosinophil CFU counts in mice with experimental asthma equal eosinophil CFU counts reported in asthmatic patients after allergen challenge [36]. Besides stimulation of eosinopoiesis, IL-5 represents a key factor in the recruitment of eosinophils towards bronchi [37]. Therefore, stimulation of IL-5 production may be one of the key mechanisms involved in the progesterone-mediated aggravation of airway eosinophilia. In addition to IL-5-mediated effects of progesterone, progesterone may also directly augment bronchial eosinophilia through its capacity to promote adhesion of eosinophils to human endothelial cells [38] and/or enhance the chemotactic capacity of eosinophils [39]. Finally, progesterone has been demonstrated to play a role in the degranulation process of eosinophils [38]. Here, we found higher levels of EPO activity in the progesterone-treated mice, suggestive of a higher activation state of airway eosinophils in the progesterone group. We should, however, acknowledge that EPO activity may also reflect traumatic rupture of eosinophils during processing [40] and thus may be a measure of the mere presence of eosinophils rather than of their activated state.

Our observation of a remarkable correlation between aggravation of airway eosinophilia, elevation of circulating levels of IL-5 and enhanced bone marrow eosinopoiesis after treatment with progesterone highlights the potentially important interplay between immunologic responses in the airways and remote organs, like the bone marrow. In spite of our finding of more eosinophil CFUs in the bone marrow and elevated bronchial eosinophilia in progesterone-treated allergen-challenged mice, similar percentages of eosinophils were found in the bone marrow and blood. These data therefore suggest a rapid transit of newly formed eosinophils out of the bone marrow towards inflamed bronchi in response to allergen inhalation.

It its noteworthy to mention that repeated administration of high doses of progesterone only slightly decreased the

levels of endogenously produced progesterone (unpublished data) and did not cause any weight change or clinically obvious side-effect. In light of the lack of alteration of endogenous progesterone levels, the observed effects reflect the effects of exogenously administered progesterone.

In contrast to aggravation of eosinophilic airway inflammation and higher IL-4 levels in the BAL fluid, progesterone did not alter IL-4 levels or allergen-specific IgE levels in the serum. These data suggest that IgE-independent mechanisms account for aggravation of allergic airway inflammation by progesterone.

Our observations in male mice are in line with some, but not all, clinical observations in female asthmatic patients. Indeed, progesterone has been reported to have asthma-promoting effects [12], whereas others reported no significant effect of progesterone on asthma severity [14]. Nevertheless, our data are consistent with *in vitro* data on the role of progesterone on the differentiation of Th cells towards the Th2 lineage and may help, in some way, to clarify premenstrual asthma exacerbation. The variable effects of progesterone probably relate to the heterogeneity of the asthmatic population and the lack of carefully conducted clinical trials on the effects of progesterone and/or oestrogen on allergic asthma. Only then will it be possible to delineate the potentially harmful or beneficial effects of female sex hormones in patients with allergic asthma. In conclusion, our data highlight the stimulatory capacity of progesterone on Th2-mediated bronchial inflammation in previously sensitized mice. Therefore, the major value of this study relates to the generation of experimental evidence for aggravation of allergic airway inflammation by progesterone and for its underlying mechanism.

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