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RESEARCH ARTICLE

Ovalbumin-Induced Chronic Asthma in Female Wistar Rats: Eosinophilic Infiltration, IL-5 Profiles and Bronchial Histopathology

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Abstract

Asthma, a chronic inflammatory airway disease, is marked by bronchial hyper responsiveness, airway inflammation, and remodeling, leading to symptoms such as wheezing, chest tightness, and cough, which may result in permanent lung function impairment. This study aimed to investigate the progressive effects of ovalbumin (OVA) sensitization on eosinophil levels, interleukin-5 (IL-5) concentrations, and bronchial histopathology in a rat model of asthma. Female Wistar rats were divided into 5 groups: A control group and 4 OVA-sensitized groups (2, 4, 6, and 8 weeks) via intraperitoneal injection and inhalation at varying frequencies. Eosinophil levels, IL-5 concentrations, and bronchial histopathological changes were assessed. Eosinophil levels and IL-5 concentrations varied significantly among groups, with OVA sensitization leading to elevated levels compared to the Normal Control (NC) group. The highest eosinophil and IL-5 levels were observed in the 6 weeks (OVA-6 group), indicating pronounced airway inflammation at 6 weeks post-OVA induction ($p < 0.05$). Histopathological analysis indicated progressive bronchial damage in OVA-exposed groups, with severe structural changes such as septal thickening and lymphocyte infiltration peaking at 6 weeks (OVA-6) and slightly reduced at 8 weeks (OVA-8). OVA sensitization induces time-dependent increases in eosinophil levels, IL-5 concentrations, and bronchial remodeling, with peak inflammation at 6 weeks. IL-5 plays a pivotal role in regulating eosinophilic activity and airway inflammation. These findings underscore the therapeutic potential of targeting IL-5 during peak inflammation to mitigate chronic asthma symptoms and prevent long-term airway remodeling.

Keywords: Chronic asthma, Eosinophil, Histopathology, Interleukin-5, Ovalbumin

Introduction

Asthma represents a global health challenge, affecting over 339 million people worldwide, with an annual mortality rate of 270,000. By 2025, this number is expected to increase to 400 million [1]. Asthma is a chronic inflammatory disorder involving a complex interplay of immune cells, including eosinophils, basophils, neutrophils, monocytes, macrophages, and activated mast cells [2]. These immune cells release inflammatory mediators, such as interleukins and cysteinyl leukotrienes, which drive the primary features of asthma, including airway hyper responsiveness and inflammation [3].

T-helper cells predominantly mediate asthma pathogenesis, specifically Th1 and Th2 responses. Th2-

driven immune activation is crucial for the proliferation and differentiation of eosinophils, which play a vital role in the inflammatory process [4,5]. Further, Th2-mediated cytokines (Interleukin/IL-4, IL-5, IL-9, and IL-13) are known to prolong and amplify this inflammatory response, worsening asthma symptoms [6,7]. Our findings align with those of Tian (2024), who reported similar reductions in IL-5 and IL-13 levels in OVA-exposed mice, further supporting the therapeutic potential of the intervention[8].

Ovalbumin (OVA), the predominant protein in egg whites, is extensively used to induce eosinophilic asthma in animal models[9], as it reliably mimics the Th2-mediated allergic inflammation observed in human asthma [10,11]. The OVA sensitization and challenge model is widely employed in asthma research, as it closely reproduces airway inflammation and allergic responses [12]. OVA induces the cross-linking of IgE antibodies on mast cells, triggering the release of key inflammatory cells such as dendritic cells, macrophages, eosinophils, and neutrophils, along with the release of Th2 cytokines like IL-4, IL-5, and IL-13 [13]. These cytokines are critical in asthma exacerbation, with IL-4 stimulating IgE production and mucus secretion. At the same time, IL-5 is crucial for eosinophil recruitment and infiltration into the airways, driving airway hyper responsiveness [14,15]. Chronic OVA exposure models the structural airway changes seen in severe human asthma, evidenced by eosinophil infiltration and other inflammatory cells [16].

Recent studies have employed the ovalbumin (OVA)-induced asthma model to investigate potential therapeutic interventions, such as plant-derived compounds, such as saffron Zhang *et al.* [17], Ephedrae Herba polysaccharides Dagan *et al.* [18], and Glabridin Azman *et al.* [19]. Most of these studies focus primarily on the efficacy of natural compounds in mitigating airway inflammation and oxidative stress. Other recent investigations have explored the effects of traditional medicinal plants Han *et al.* [20] and agents like Paeoniflorin Lin *et al.* [21] and lactoferrin Maltby *et al.* [22] on OVA-induced asthma. However, most of these studies are conducted in male rodents, and few provide comprehensive insights into the sex-specific immune responses, particularly eosinophilic infiltration and interleukin-5 profiles in female models. Moreover, research focusing on female-specific asthma pathophysiology remains scarce despite known sex differences in immune responses to allergens. To the best of our knowledge, no study has specifically addressed eosinophilic infiltration, IL-5 levels, and bronchial histopathology in female Wistar rats subjected to OVA-induced asthma.

This study investigates the time-dependent outcomes of ovalbumin (OVA) sensitization and exposure in female Wistar rats, focusing on eosinophil infiltration, interleukin-5 (IL-5) concentrations, and bronchial remodeling. Wistar rats were chosen for their suitability in chronic asthma models, particularly for studying airway remodeling and inflammation. This study builds upon prior research using murine models, aiming to identify temporal patterns of disease progression and to assess the suitability of rats as a complementary model for understanding allergic airway diseases. These findings provide insights into the temporal dynamics of allergic airway disease and their implications for therapeutic intervention.

Materials and methods

Animals

Female Wistar rats (*Rattus Norvegicus*), aged 6-8 weeks and weighing 170 - 200 g, were obtained from the Integrated Research and Testing Laboratory Unit 4, Universitas Gadjah Mada, Yogyakarta. The experimental protocol for asthma modeling in these rats was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (Ethical no: 111/EC-FKH/int./2024). All procedures followed the previous studies protocol [16,23-27]. Rats were housed in a controlled environment with regulated lighting (12/12 h light/dark cycle), temperature (22 ± 1 °C), and humidity (55 ± 5 %) and provided free access to a standard pellet diet and water ad libitum. One week before to the research, the animals were acclimated. Sample size calculation was based on the Degree of Freedom formula, with a minimum of 3 rats for each group.

Chemicals

Ovalbumin (Grade V, purity > 98 %, Sigma, St. Louis, USA) was employed as the asthma allergen. Aluminum hydroxide ($Al(OH)_3$) was also purchased from Sigma Chemicals, St Louis, USA. Ketamine and

xylazine used for anesthesia were sourced from Troy Laboratories (Australia) and Interchemie (Holland).

Experimental design

The rats were divided into 5 groups, i.e.:

Group 1 (Normal Control, NC): Healthy female Wistar rats without treatment.

Group 2 (OVA-2): Female Wistar rats induced with ovalbumin for 2 weeks.

Group 3 (OVA-4): Female Wistar rats induced with ovalbumin for 4 weeks.

Group 4 (OVA-6): Female Wistar rats induced with ovalbumin for 6 weeks.

Group 5 (OVA-8): Female Wistar rats induced with ovalbumin for 8 weeks.

Female Wistar rats were sacrificed under anesthesia using ketamine (100 mg/kg) and xylazine (15 mg/kg). Blood and tissue samples were collected for further analysis.

Study protocol

Female Wistar rats were divided into 4 groups, each receiving different durations of ovalbumin (OVA) treatment: 2, 4, 6, and 8 weeks to model chronic asthma (**Figure 1**). For sensitization, rats received an intraperitoneal injection of 75 µg OVA adsorbed in 75 mg/mL aluminum hydroxide [$Al(OH)_3$] on days 0, 7, and 14. Following this, aerosolized 1 % OVA (in saline) was administered for 30 min on alternate days using a nebulizer (flow rate: 0.22 mL/min). In the 2-week protocol (**Figure 1(a)**), rats were induced for 14 days. In the 4-week (**Figure 1(b)**), 6-week (**Figure 1(c)**), and 8-week (**Figure 1(d)**) protocols, the aerosol challenge continued for the respective duration post-sensitization. At the end of each designated period, rats were anesthetized using ketamine (100 mg/kg) and xylazine (15 mg/kg), and blood and lung tissues were collected for eosinophil counts, interleukin-5 (IL-5) measurements, and histopathological analysis of eosinophilic infiltration in bronchial tissues.

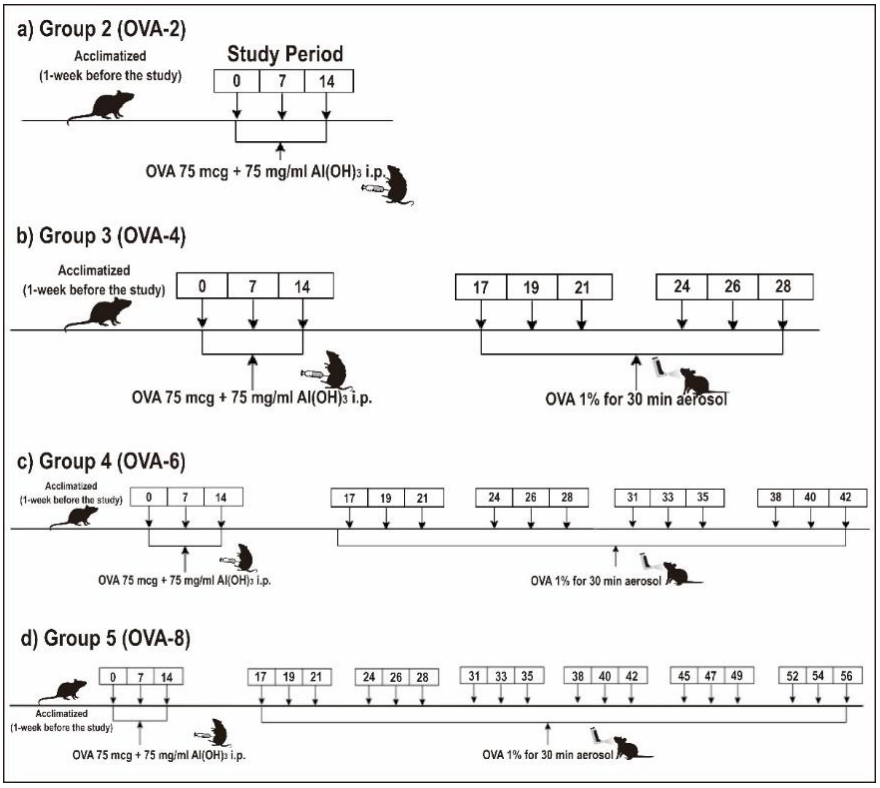

Figure 1 Study protocol for OVA-induced asthma in rats. (a) Group 2 (OVA-2) sensitized with 75 µg OVA + 75 mg/mL $Al(OH)_3$ i.p. on days 0, 7, and 14 (2-week study). (b) Group 3 (OVA-4) received the same sensitization with OVA aerosol (1 %) on days 24, 26, and 28 (4-week study). (c) Group 4 (OVA-6) had additional OVA aerosol on days 24, 26, 28, 31, 33, 35, 38, and 40 (6-week study). (d) Group 5 (OVA-8) extended aerosol exposure until day 56 (8-week study). All groups were acclimatized for 1 week.

Blood sampling and serum isolation

Blood samples were collected from the orbital vein at room temperature. Samples were left to clot for 30 min and centrifuged at 1000 g for 15 min. The serum was separated and stored at temperature −80 °C for the quantitative determination of cytokines [28]. Serum interleukin-5 (IL-5) levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit, and plates were analyzed using an automated plate reader (Zhejiang, China).

Eosinophil and IL-5 levels

After the initial sensitization with ovalbumin and aluminum hydroxide, rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Following anesthesia, the rats were intratracheally intubated, and bronchoalveolar lavage fluid (BALF) samples were collected to measure eosinophil levels and IL-5 concentrations [27,29]. After intubation, rats were exposed to aerosolized ovalbumin (1 % in sterile water) using a nebulizer at an airflow rate of 10 L/min for 30 min. Eosinophil counts in BALF were determined using a hemocytometer, while IL-5 concentrations were measured through enzyme-linked immunosorbent assay (ELISA). These assessments were repeated at specific intervals throughout the exposure period to monitor the progression of inflammatory response across different treatment groups.

Histopathological analysis

Dissected lung tissues were preserved in 10 % formaldehyde after being perfused with 0.9 % NaCl. The fixed lung specimens were sectioned and stained with hematoxylin and eosin (H&E). Periodic acid-Schiff (PAS) staining was used to assess mucus hypersecretion. Histological analysis focused on bronchial inflammation, structural remodeling, and eosinophilic infiltration in bronchial tissues. Selected sections were imaged at 200× magnification for analysis.

Statistical analysis

All experiment's data was presented as mean ± Standard Deviation (SD). SPSS version 29.0 for Windows was used to conduct the statistical analysis. Eosinophil counts were tested for normality using the Shapiro-Wilk test. Data with non-normal distributions were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons. Serum IL-5 levels, which were normally distributed, were analyzed using one-way analysis of variance (ANOVA) to assess differences amongst multiple groups, followed by Tukey's post-hoc test. Histopathological comparisons of bronchial tissues between the control and ovalbumin-induced groups were made to determine asthma-like pathology. While a formal power analysis was not conducted using specific statistical software, the sample size was determined based on prior studies employing similar methods in the field of asthma research. The chosen sample size was sufficient to detect statistically significant differences in the measured parameters across experimental groups. A p-value of < 0.05 was considered statistically significant.

Results and discussion

Normality test

Eosinophil levels varied significantly across groups. **Table 1** shows the normality test results for eosinophil levels and IL-5 concentrations. The eosinophil data for the Normal Control (NC) group did not yield a p-value due to identical values (all zero). For the OVA-4 group (Group 3), the p-value was < 0.05, indicating a non-normal distribution, whereas the OVA-2 (Group 2), OVA-6 (Group 4), and OVA-8 (Group 5) groups obtained p-values > 0.05, indicating normally distributed data. Given that 1 group had a non-normal distribution, the Kruskal-Wallis test was used to compare eosinophil levels across all groups.

Table 1 Normality test for eosinophil and IL-5 levels.

Group	*p-value*	
	Eosinophil	**IL-5**
NC	-	0.557
OVA-2	1.000	0.644
OVA-4	0.017	0.096
OVA-6	0.395	0.944
OVA-8	0.122	0.441

Eosinophil Level

Table 2 summarizes the eosinophil levels across groups based on Kruskal-Wallis analysis (p-value = 0.025). The NC group revealed the lowest mean eosinophil level (0.00 ± 0.00, $p < 0.05$), significantly lower than all OVA-induced groups. Among OVA-treated groups, the OVA-6 group showed the highest eosinophil level (1.53 ± 0.19, $p < 0.05$), while OVA-2, OVA-4, and OVA-8 groups displayed intermediate levels without significant differences between them.

Table 2 Comparison of eosinophil levels.

Group	Mean ± SD	*p*-value (Kruskal-Wallis)
NC	0.00 ± 0.00^{a}	0.025
OVA-2	0.27 ± 0.20^{b}	
OVA-4	0.49 ± 0.56^{b}	
OVA-6	1.53 ± 0.19^{c}	
OVA-8	0.38 ± 0.16^{b}	

Note: Superscript letters indicate results from the Mann-Whitney test. Groups with different letters are significantly different ($p < 0.05$).

The histogram of mean eosinophil levels across groups illustrates that the NC group exhibited the lowest eosinophil levels, while the OVA-6 group obtained the highest. The OVA-2, OVA-4, and OVA-8 groups showed moderate eosinophil levels (see **Figure 2**).

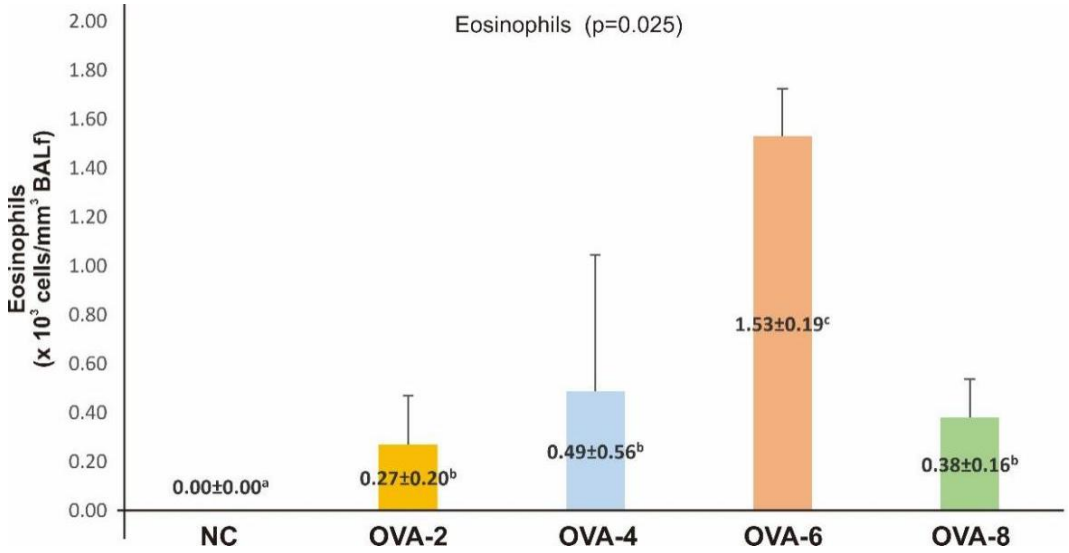

Figure 2 Eosinophil levels (×10³ cells/mm³ BALf) across different treatment groups. Bars represent mean ± SD of eosinophil counts in bronchoalveolar lavage fluid (BALf) from 5 experimental groups: NC (negative control), OVA-2, OVA-4, OVA-6, and OVA-8. Statistical significance was analyzed with a *p*-value of 0.025. Different superscripts (a, b, c) denote statistically significant differences between groups, with each letter indicating distinct groupings ($p < 0.05$).

Interleukin (IL)-5 level

One-way ANOVA analysis indicated significant differences in IL-5 levels among the groups (*p*-value = 0.000). It was followed by a post hoc Tukey's test to compare IL-5 levels between specific groups (**Table 3**).

Table 3 Comparison of IL-5 levels (ng/L).

Group	Mean ± SD	*p*-value (ANOVA)
NC	48 ± 35^{a}	0.000
OVA-2	26770 ± 13072^{b}	
OVA-4	47321 ± 13110^{bc}	
OVA-6	86327 ± 4221^{d}	
OVA-8	54475 ± 8016^{c}	

Note: Superscript letters indicate the results of Tukey's post hoc test; different letters denote significant differences ($p < 0.05$), while shared letters indicate non-significant differences ($p > 0.05$).

Tukey's test demonstrated significant differences in mean IL-5 levels between the Normal Control (NC) group ($p = 0.000$). The NC group exhibited the lowest IL-5 levels (48 ± 35^{a} ng/L) while OVA-induced groups. The IL-5 levels in the NC group were the lowest among all groups. The mean IL-5 levels in the OVA-2 group (26770 ± 13072^{b} ng/L) were significantly higher than in the NC group but did not differ significantly from the OVA-4 group (47321 ± 13110^{bc} ng/L). However, the OVA-2 group differed significantly from the OVA-6 group (86327 ± 4221^{d} ng/L) and the OVA-8 group (54475 ± 8016^{c} ng/L). The OVA-4 group discovered an increase in IL-5 levels compared to OVA-2, with levels peaking in the OVA-6 group. A decline in IL-5 levels was observed in the OVA-8 group, yet levels remained significantly higher than in the NC group. Significant differences were also observed between the OVA-4 and OVA-6 groups ($p < 0.05$), while the OVA-4 and OVA-

8 groups did not differ significantly. The OVA-6 group had the highest mean IL-5 level, significantly different from all other groups, including the OVA-8 group.

The histogram of mean IL-5 levels (**Figure 3**) illustrates the distribution across groups, with the NC group showing the lowest IL-5 levels and the OVA-6 group displaying the highest levels. The trend in IL-5 levels suggests an increase up to 6 weeks post-OVA induction, followed by a decrease at 8 weeks.

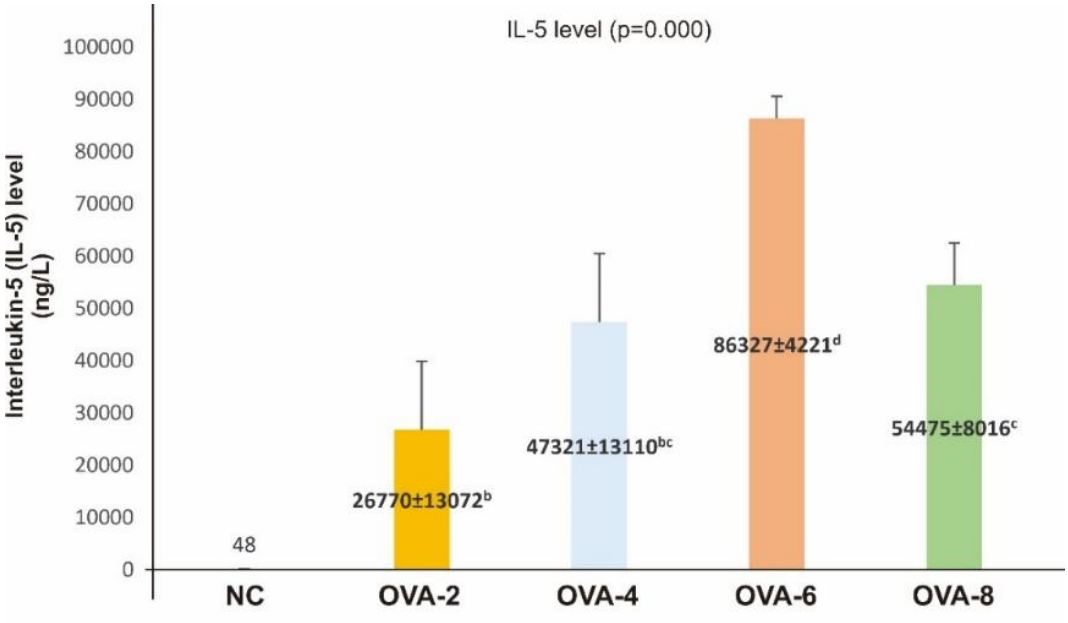

Figure 3 Levels of Interleukin-5 (IL-5) in different treatment groups. IL-5 concentrations (ng/L) are displayed for the normal control (NC) group and the ovalbumin (OVA)-sensitized groups at various exposure durations (OVA-2, OVA-4, OVA-6, OVA-8). Data are presented as mean ± SD, with values rounded to 2 decimal places for consistency (e.g., 0.557 → 0.56, 0.644 → 0.64, 34.66 remains unchanged, 26770 ± 13072 → 267.60 ± 130.72). Statistical significance ($p = 0.000$) was observed among the groups, with different letters (b, bc, d, c) indicating statistically significant differences ($p < 0.05$) according to post hoc analysis.

Bronchial histopathology

Histopathological analysis revealed progressive bronchial damage with increased OVA exposure time (**Figure 4**).

The Normal Control (NC) group showed that the bronchial and alveolar structures appeared normal with an intact architectural structure, and were assigned a pathology score of 0. After 2 weeks (OVA-2 group), minimal changes were observed, with a score of 1. In the OVA-4 group, histopathological analysis showed thickening of the interalveolar septa and alveolar interstitial, accompanied by lymphocyte infiltration and type II pneumocyte hyperplasia. However, the bronchial architecture remained largely intact, with a pathology score of 1. The OVA-6 group exhibited the most severe structural alterations, including marked septal thickening, pronounced lymphocyte infiltration, and hyperplasia, resulting in the highest pathology score of 2. In the OVA-8 group, pathological features similar to those observed in the OVA-6 group were present, but the severity of bronchial damage appeared slightly reduced, yielding a pathology score of 1. **Figure 4** demonstrates that histopathological damage began to emerge at 2 weeks of OVA exposure (OVA-2), with pronounced structural deterioration occurring at 6 weeks (OVA-6). The inflammation progressively increased with longer sensitization periods. The OVA-6 group showed the highest inflammation score (48.8), indicating that 6 weeks of OVA sensitization induced the most pronounced bronchial inflammation (**Figure 5**). After 8 weeks (OVA-8), although pathological changes were still evident, the extent of bronchial damage was less severe than at 6 weeks. The higher eosinophil counts in the 6-week group compared to the

8-week group due to the resolution of acute inflammation at later time points, as the immune response transitions toward chronic remodeling [30]. This phenomenon has been observed in other chronic asthma models, where peak eosinophilic inflammation occurs before the onset of extensive tissue remodeling.

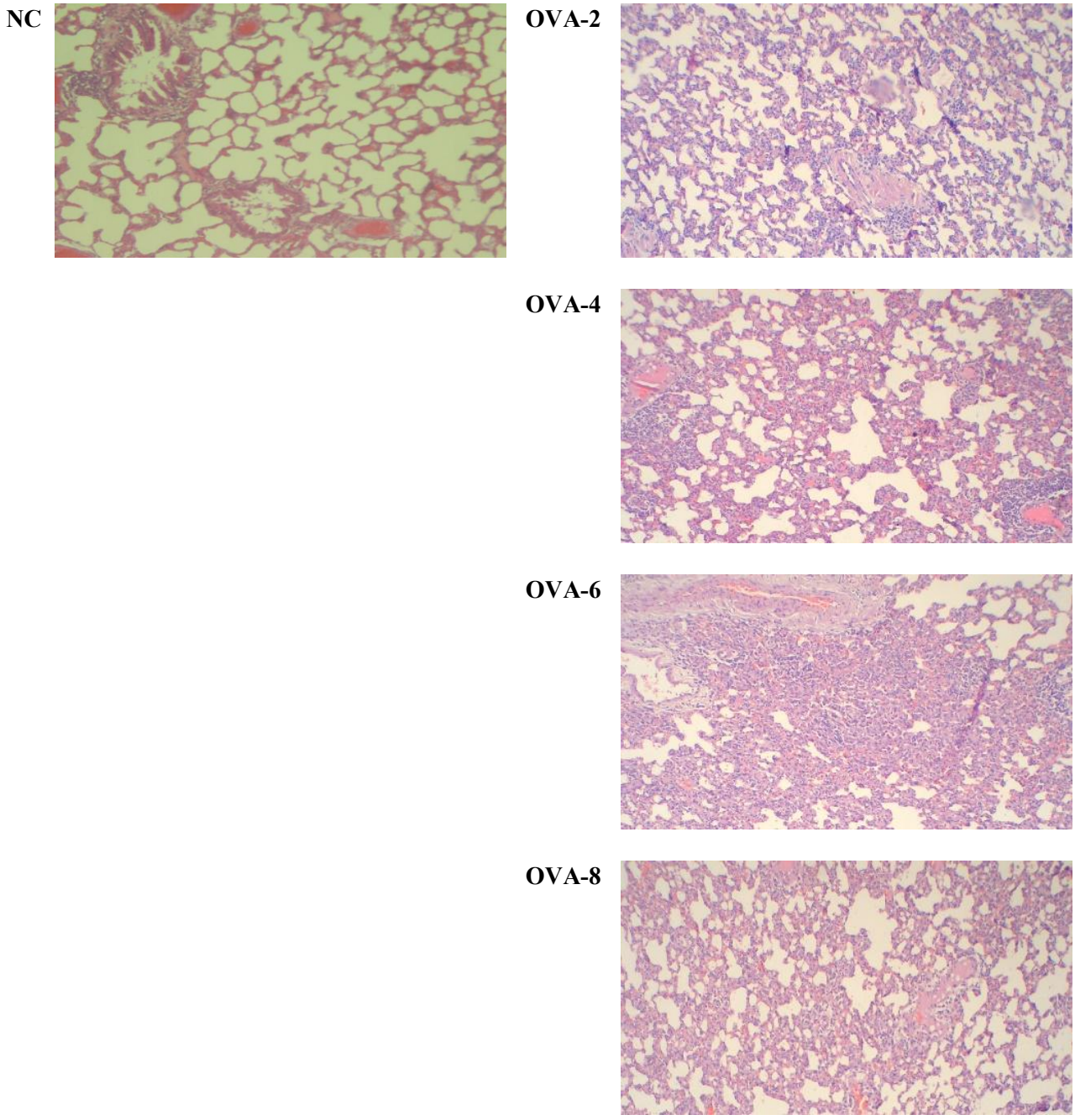

Figure 4 Histopathological analysis of lung tissues from the normal control (NC) group and ovalbumin (OVA)-sensitized groups at different exposure durations (OVA-2, OVA-4, OVA-6, OVA-8). Hematoxylin and eosin (H&E) staining reveals inflammatory cell infiltration and structural changes in lung tissues, with varying degrees of severity across the OVA-exposed groups. The NC group exhibits normal lung architecture, whereas the OVA-2, OVA-4, OVA-6, and OVA-8 groups show interalveolar septal thickening, lymphocyte infiltration, and type II pneumocyte hyperplasia. The most pronounced pathological changes are observed in the OVA-6 group. Images were captured at 200× magnification (20× objective lens, 10× ocular lens).

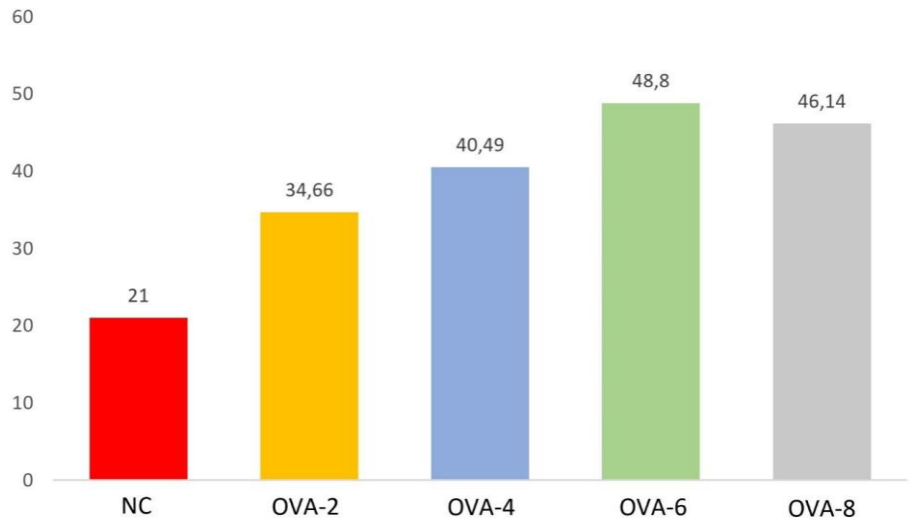

Figure 5 Mean bronchial inflammation was lowest in the normal control (NC) group, indicating minimal inflammation in rats without ovalbumin sensitization. The highest mean inflammation was observed in the OVA-6 group, highlighting that 6 weeks of ovalbumin sensitization induced the most significant bronchial inflammation compared to other groups**.**

Moreover, one-Way ANOVA test indicated that the normal control (NC) group did not exhibit any signs of bronchial inflammation. In contrast, significant bronchial inflammation was observed in the ovalbumin (OVA)-sensitized groups at different time points, with a p-value < 0.05 (see **Table 4**).

Table 4 Comparison of bronchial inflammation across groups.

Observation Group	Mean ± SD	*p*-value (One-Way ANOVA)
NC	21.00 ± 1.00	0.003
OVA-2	34.66 ± 3.46	
OVA-4	40.49 ± 13.33	
OVA-6	48.80 ± 3.64	
OVA-8	46.14 ± 2.41	

To the best of our knowledge, this is the first study to comprehensively examine the progressive impact of ovalbumin (OVA) sensitization on eosinophil levels, interleukin-5 (IL-5) concentrations, and bronchial histopathology in a time-dependent manner. Our results provided novel insights into the inflammatory processes underlying OVA-induced asthma-like pathology, particularly about eosinophilic activity and IL-5 expression, as well as structural changes within bronchial tissue over time. These findings offered significant implications for understanding the pathophysiology of asthma and the temporal progression of airway inflammation, potentially guiding future therapeutic interventions targeting chronic allergic airway disease.

The eosinophil levels observed in this study reflected the hallmark eosinophilic inflammation associated with asthma [31]. Eosinophils play a critical role in mediating allergic reactions and are well-known to contribute to airway hyper-responsiveness, tissue

remodeling, and mucus hypersecretion[31,32]. Our results demonstrated a marked increase in eosinophil levels in all OVA-treated groups compared to the Normal Control (NC) group, with the highest levels observed in the OVA-6 group. This peak aligns with the most severe histopathological damage in bronchial tissue, including pronounced septal thickening, lymphocyte infiltration, and type II pneumocyte hyperplasia [16,33]. The correlation between elevated eosinophil levels and bronchial damage [34,35] underscored the pivotal role of eosinophils in driving and sustaining airway inflammation and suggested a possible threshold or tipping point in the inflammatory response around the 6-week mark, indicating a critical threshold in the inflammatory response.

Interestingly, while eosinophil levels decreased slightly by the 8 weeks (OVA-8 group), they remained significantly higher than in the NC group (**Figure 5**). This decrease could indicate a potential down-regulation of the inflammatory response over prolonged exposure or may reflect adaptive changes within the immune response [33,35]. Similar trends were observed in chronic inflammation models, where prolonged antigen exposure could lead to immune tolerance or modulation of the inflammatory response [36]. This finding suggested a potential avenue for future research exploring the long-term effects of sustained allergen exposure and possible mechanisms of immune adaptation.

IL-5 concentrations varied significantly across groups, paralleling eosinophil counts and underscoring IL-5's role in eosinophilic regulation. IL-5 drives eosinophil differentiation, survival, and migration, making it crucial in allergic asthma pathogenesis [33,37]. The OVA-6 group displayed peak IL-5 levels, correlating with the highest eosinophil counts and bronchial damage severity [38]. IL-5 strongly cooperates to induce eosinophil development and functional activation. IL-5 also inhibits eosinophil apoptosis, and sputum IL-5 levels were reported to be negatively correlated with apoptotic eosinophils in subjects with either asthma exacerbations or stable disease [39].

Moreover, in T2-high asthma, IL-5 induced eosinophil adhesion to and migration in the extracellular matrix by favoring the interaction of eosinophils with periostin, a matricellular protein whose enhanced expression was associated with eosinophil trafficking toward bronchi [40]. IL-5 was also involved in the pathobiology of late-onset, nonallergic eosinophilic asthma [41]. In this case, ILC2 and not Th2 lymphocytes were mainly responsible for IL-5 production [42]. Differently from blood and airway pro-inflammatory eosinophils, the lung resident subsets of homeostatic anti-inflammatory and anti-allergic eosinophils seemed to be partially independent of IL-5, at least in mice [43]. Our findings indicate that IL-5-driven eosinophilic inflammation was most pronounced in the OVA-6 group, suggesting a potential peak response at this exposure duration.

Moreover, IL-5 levels declined in the OVA-8 group, mirroring the reduction in eosinophil counts and bronchial damage severity. This pattern could indicate a regulatory feedback mechanism to modulate excessive inflammation to prevent further tissue damage. Previous studies showed that chronic exposure to allergens could lead to desensitization or alteration in cytokine profiles, which might explain the decreased IL-5 at later stages [38]. However, despite this reduction, IL-5 levels in the OVA-8 group remained significantly higher than in the NC group, highlighting the persistence of inflammation even after prolonged allergen exposure [44]. These findings underscored the potential of targeting IL-5 as a therapeutic strategy, especially during peak inflammatory phases, to effectively control eosinophil-mediated pathology [45].

Histopathological examination revealed progressive structural changes within the bronchial tissue of OVA-sensitized groups, supporting the findings from eosinophil and IL-5 assessments. The NC group displayed intact bronchial architecture, while the OVA groups exhibited varying degrees of inflammation and structural remodeling. At 2 weeks (OVA-2), mild pathological changes were observed, including minimal lymphocyte infiltration and early signs of pneumocyte hyperplasia. By 4 weeks (OVA-4), these changes intensified, with thickening of the interalveolar septa and more pronounced lymphocyte infiltration, indicating escalating inflammation. The most severe structural damage was observed in the OVA-6 group, where extensive lymphocyte infiltration, marked septa thickening, and hyperplasia of type II pneumocytes were present [35]. These changes were characteristic of airway remodeling in chronic asthma, where persistent

inflammation led to structural alterations contributing to airway obstruction and hyper-responsiveness [37]. The slight reduction in histopathological severity observed in the OVA-8 group aligns with the decrease in eosinophil and IL-5 levels, suggesting that some degree of spontaneous repair or immune adaptation may occur with prolonged allergen exposure [39].

The critical role of eosinophils and IL-5 in driving airway inflammation and remodeling in OVA-induced asthma models was also important based on our findings. The temporal dynamics observed, particularly the peak inflammatory response at 6 weeks, provided valuable insight into the progression of allergic airway disease and suggested that early intervention during peak inflammatory phases could be most effective in managing chronic asthma. Targeting IL-5 specifically, shown to regulate eosinophilic activity directly, presented a compelling therapeutic strategy [38,44]. IL-5 inhibitors, such as mepolizumab and reslizumab, effectively reduced eosinophilic inflammation in patients with severe asthma [33]. Our findings further support the potential of IL-5-targeted therapies Kusmardi *et al.* [45], especially when administered during peak inflammatory phases, to mitigate structural airway changes and improve clinical outcomes. Additionally, the observed decline in inflammatory markers and bronchial damage at 8 weeks suggests a potential window for secondary intervention aimed at promoting tissue repair and preventing further remodeling. Therapies focused on modulating immune response, such as immunotherapy Nagata and Nakagome [43] or allergen-specific desensitization [17], might offer benefits in reducing the long-term impact of chronic allergen exposure and limiting irreversible airway remodeling [46].

While this study provided significant insights, some limitations should be acknowledged. First, the sample size in each group was relatively small, and additional studies with larger sample sizes were necessary to confirm these findings. Furthermore, this study did not explore molecular mechanisms underlying IL-5-mediated eosinophilic activation or bronchial remodeling in detail. The findings are based on an animal model and may not fully translate to human physiology. Further studies are needed to validate these results in clinical settings and should focus on elucidating specific signaling pathways and regulatory factors contributing to the observed temporal changes in inflammation and remodeling. Additionally, long-term studies examining immune adaptation or tolerance in response to chronic allergen exposure could provide further insights into potential therapeutic strategies for managing chronic allergic airway diseases.

Conclusions

This study demonstrates that OVA sensitization induces time-dependent increases in eosinophil levels, IL-5 concentrations, and bronchial remodeling, with peak inflammatory response occurring around 6 weeks post-sensitization. The findings highlight IL-5's critical role in mediating eosinophilic activity and suggest that interventions targeting IL-5 during peak inflammation may effectively mitigate chronic asthma symptoms and prevent airway remodeling. These results provide a foundation for the development of targeted therapies that can address the inflammatory mechanisms underlying asthma and related allergic disorders. This study contributes to the advancement of asthma treatments by highlighting specific therapeutic windows during peak inflammation, offering insights that could enhance the timing and precision of existing therapies. Furthermore, these findings have the potential to refine current approaches by complementing established treatments and addressing limitations in their efficacy. Future research should focus on understanding the long-term immune adaptations associated with OVA sensitization and conducting clinical studies to evaluate the effectiveness of these therapeutic strategies in diverse and representative patient populations. This comprehensive approach will help ensure broader applicability and improved outcomes in asthma management.

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