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Studying the Effects of Vitamin A on the Severity of Allergic Rhinitis and Asthma

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ABSTRACT

Allergic asthma is a complex lung disease characterized by breathlessness, airway inflammation, and obstruction. Allergy and allergic rhinitis (AR) are the main triggers of asthma. Vitamin A is an important supplementary factor for the physiological activation of the immune system. In the present study, we investigated the effects of vitamin A on the exacerbation of allergic asthma symptoms.

BALB/c mice were allocated to four groups. Asthma was created in two groups, and in the other two groups, rhinitis was induced. One of the asthma groups and one of the rhinitis groups orally received vitamin A (20 IU/g for 15 days). The levels of Immunoglobulin (Ig) E, histamine, leukotriene B4 (LTB4), Cysteinyl leukotriene receptor (Cys-LT), interleukin (IL)-4, IL-5, IL-13, and IL-35 as well as eosinophil peroxidase activity, were measured. Also, the histopathology of mice lungs was evaluated.

The levels of total IgE, LTB4, Cys-LT, IL-4, IL-5, IL-17, and IL-33, eosinophil peroxidase activity, perivascular and peribronchial inflammation significantly decreased in vitamin A-treated asthma and rhinitis groups compared to non-treated groups. Also, IL-13 and histamine levels, hyperplasia of the goblet cell, and hyper-secretion of the mucus insignificantly decreased in vitamin A-treated asthma and rhinitis groups.

Asthma and AR are common diseases that are generally developed due to the dysregulation of the immune system. Vitamin A plays an important role in controlling the immunopathologic mechanisms of allergic diseases. Vitamin A could be a useful supplement in managing AR and asthma by decreasing the severity of inflammatory responses. Therefore, control of vitamin A deficiency is recommended in Allergy.

Keywords: Allergy and immunology; Asthma; Lung; Vitamin A

INTRODUCTION

Asthma as a complicated disease of bronchi is characterized by dyspnea, cough, and wheezing. Regarding immunopathologic features, asthma is associated with eosinophilic infiltration around bronchi

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and vessels, airway smooth muscle spasm, AHR, mucus hyper-secretion, goblet cell hyperplasia, reversible obstruction of airflow, and remodeling of the airway.[1,2]

More than 350 million people were affected by asthma worldwide and causes more than 250000 deaths per year. Asthma occurs in all ages and is triggered by allergic diseases of the respiratory tract such as AR. The current treatment protocols cannot completely cure asthma. Predisposition to asthma is influenced by the genetic and environmental factors that modulate the immune system. Allergic reactions are important in the development of asthma.[3,4] Allergy is mediated by Th2 cells that produce the cytokines (e.g., IL-4, 5, and 13) leading to IgE production, atopic reactions, bronchoconstriction, eosinophilic airway inflammation, and mucus hyper-secretion.[1,2,4]

Some vitamins are present in dietary supplements can influence the development of allergy by controlling related immunopathologic mechanisms. Vitamin A is a fat-soluble beta-carotenoid and the basic molecule of retinol. Vitamin A is captured through the diet. Preformed vitamin A is derived from animal sources, and pro-vitamin A or beta-carotenoid is derived from plants. Its metabolites are essential for cell differentiation, vision, immune system function, metabolism, and reproductive functions. Vitamin A deficiency is a common cause of blindness and ocular symptoms. There is inadequate research on the effects of Vitamin A on allergy and asthma pathophysiology. Vitamin A has antioxidant activity; however, its role in the development of allergy has not been completely explained.[5-7] It was reported that vitamin A deficiency was associated with respiratory system diseases, and the fact that this vitamin has a role in maintaining the integrity of the lung epithelium along with its anti-inflammatory and anti-emphysematous effects.[8,9]

Vitamin A, as a multifunctional vitamin, is involved in several essential processes of immune system function and antioxidant pathways.[10] The metabolites of this vitamin exert a broad range of biological activities compassing gene expression regulation (over 500 genes) via regulating a superfamily of nuclear-specific receptors.[10,11] Vitamin A deficiency increases the morbidity and mortality of respiratory tract diseases and is involved in the proliferation of respiratory tract epithelial cells, lung maturation,[12-14] as well as histopathological events in pulmonary epithelial, parenchyma, and respiratory diseases. Vitamin A is also required for alveolar architecture maintenance and regeneration of tissue.[15-17] Nevertheless; there are a few studies on the effects of vitamin A on bronchi. Therefore, in this study, we aimed to study the effects of vitamin A supplementation on asthmatic airway properties and related immunopathological factors.

MATERIALS AND METHODS

Animals

Female BALB/c mice (6-8-week old, 23±2 gr weight) were purchased from the Pasteur Institute of Iran and allowed to be acclimatized under pathogen- and allergen-free standard laboratory conditions (25±2°C temperature, 60±5% humidity, 12h light-dark cycle, and also free access to water and food).

The animals' studies were approved by the ethics committee of ix.med.vet.dep, animal house (No. IX.MED.VET.DEP.REC.2020.150009.3).

Animal Sensitization and Treatment Schedule

Fifty mice were allocated to5groups (n=10, five mice for bronchoalveolar lavage fluid (BALF) sampling and the other five for histopathological studies) including the healthy mice that received no treatment (control), asthmatic control mice that received no treatment, asthmatic animals treated with vitamin A, AR control mice that received no treatment, and finally, AR mice treated with vitamin A. In two groups, airway allergic inflammation was induced by ovalbumin (Sigma-Aldrich, USA), and in two other groups, AR was induced by administering ovalbumin (OVA) according to a previously described protocol.[4,18-20] Briefly, for inducing AR, 0.3 mg OVA and 30 mg aluminum hydroxide powder (alum adjuvant) were suspended in 1 mL of normal saline and intraperitoneally (i.p.) injected every day for seven days. At the next stage, 2 mg OVA in 20 µL normal saline was daily instilled via IT for 7 days. Daily intranasal OVA administration is necessary to maintain allergic signs (until 30 days).[18,19] Nasal rubbing (nasal itching), rhinorrhea, sneezing, and congestion are useful signs indicating the induction of AR. To create an allergic asthma murine model, the mice were sensitized via the IP injection of 20 µg OVA along with 50 µL alum dissolved in 1 mL normal saline on day 1st, followed by a boosting dose on day 14th. Then the mice were challenged via IT administration of 1% OVA solution aerosolized for 30 min/day by a nebulizer on

days 24, 26, 28, and 30.[4] One asthmatic and one AR group received vitamin A (20 IU/g) by oral administration via food,[21] on days 15 to 30. Asthmatic and AR control groups received a normal diet (without vitamin A supplementation). At last, blood, BALF, and lung tissue samples were collected on day 31 after the mice were euthanized by CO_2.[4,20] Therefore, the duration of this study was 31 days.

Serum IgE Level

Blood samples were collected, and after serum separation, the total IgE level was measured by an ELISA kit (BD Biosciences, USA).

Eicosanoid Levels

On day 31, after anesthetization, five mice in each group were tracheotomized, and a catheter was placed in the trachea. BALF was collected by washing the lung with PBS (1 mL). After centrifugation of samples, the supernatant was separated and stored at −70°C before determining the levels of inflammatory mediators. Cellular infiltration was also assessed by preparing histological smears. BALF supernatant was assayed in duplicate for Cys-LT and LTB4 using ELISA kits (Cayman Chemical, Ann Arbor, USA) according to the instructions of the manufacturer.

Determining Cytokines in BALF

The IL-4, IL-5, IL-13, and IL-35 levels in BALF were measured using an enzyme immunoassay method as described before.[4]

Eosinophil Peroxidase Activity

EPO activity in BALF was determined as previously mentioned.[22] Briefly, 1 Ml of a substrate solution containing 0.1% Triton X-100,0.1 mM O-phenylenediamine dihydrochloride (OPD; Sigma), and hydrogen peroxide (1 mM) in 0.05 M tris (hydroxymethyl) aminomethane (Tris)-HCl (pH 8.0) was added to BALF supernatant (1 mL) in microtiter plates that were incubated at 37°C for 30 min. at least, the reaction was stopped by adding 4 M sulphuric acid (0.5 mL), and the absorbance was read at 492 nm.

Histamine Level

The histamine level was determined in plasma. The blood samples were collected after the last challenge and after separating plasma, the level of histamine was measured by an ELISA Kit (Biocompare, USA).

Histopathology

After euthanizing the mice, the left side of the lung was isolated and fixed with a formalin solution to prepare histopathological sections. The sections were stained with AB and H&E stains. Afterward, peri-bronchiolar inflammation, hyperplasia of the goblet cell, and hyper-secretion of the mucus in airways were evaluated using a point scoring system as described before.[4] Briefly, lung tissue sections were examined over10 randomly selected microscopy fields in five repeats. Goblet cell hyperplasia was quantified by calculating the goblet cell index. Moreover, peri-bronchiolar inflammation was examined based on the presence of incomplete or complete layers of eosinophils around the airways. Mucus hypersecretion was evaluated by ascertaining the percentage of the airways obstructed by mucus.

Statistical Analysis

Results were reported as means±SD from the experiments that were repeated three times. SPSS (version 19) was used for statistical analyses, and graphs were drawn; using GraphPad Prism (version 6). Differences between treated and non-treated groups were analyzed using the Student t-test. Also, a *p*-value of less than 0.05 was regarded to be statistically significant.

RESULTS

Serum IgE Level

Total IgE level significantly increased in non-treated asthma and rhinitis groups (2199.85±14.36 and 2497.32±32.31 ng/mL, respectively) compared to the healthy group (302.51±19.94 ng/mL) ($p<0.001$). On the other hand, total IgE level significantly decreased in the rhinitis and asthma groups that were treated with Vitamin A (1336.11±29.50 and 1401.93±20.08 ng/mL, respectively) compared to non-treated asthma and non-treated rhinitis groups (Figure 1A, $p<0.001$).

Eicosanoid Levels

The level of LTB4 in BALF significantly increased in non-treated asthma and rhinitis groups (89.2±0.20 and 96.5±0.20 pg/mL, respectively) compared to the healthy group (28.94±0.58 pg/mL) ($p<0.001$). The level of LTB4significantly decreased in Vitamin A-treated asthma and rhinitis groups (58.3±0.60 and 62.3±1.06 pg/mL, respectively) compared to non-treated asthma and rhinitis groups ($p<0.001$).

The Cys-LT level in BALF significantly increased in non-treated asthma and rhinitis groups (794.2±1.99 and 814.7±3.97 pg/mL, respectively) (p<0.001) compared to the healthy group (87.93±1.03 pg/mL). In vitamin A-treated asthma and rhinitis groups, Cys-LT level in BALF significantly decreased (126.6±1.14 and 146.6±2.84 pg/mL, respectively) compared to non-treated asthma and non-treated rhinitis groups (p<0.001) (Figure 1B).

Cytokines Levels

The levels of the studied cytokines in non-treated asthma and rhinitis groups were as follows (pg/mL): IL-4; 90.14 ±4.18 and 82.64±3.11, IL-5: 74.21±1.99 and 70.21±1.76, IL-13: 140.39±3.25 and 148.24±2.94, IL-17: 187.02±1.89 and 128.11±6.18, and IL-33: 387.25±4.15 and 379.99±8.16, respectively, (p<0.05) that showed significant increments compared with the healthy group (IL-4: 37.87±2.54, IL-5: 46.93±1.02, IL-13: 91.03±2.99, IL-17: 51.23±4.28, and IL-33: 117.36±6.20 pg/L. In the vitamin A-treated asthma and rhinitis groups, the levels of IL-4 (67.54 ±2.98 and 48.39±15.74 pg/mL) (Figure 2A), IL-5 (50.85±2.23 and 53.85±7.14 pg/mL) (Figure 2B), IL-17 (55.24±2.09 and 63.87±3.69 pg/mL) (Figure 2C) and IL-33 (174.25±3.64 and 184.45±7.96 pg/mL) (Figure 2D) significantly decreased as compared to non-treated asthma and non-treated rhinitis groups (p<0.05) (Figure 2). Comparing treated and non-treated groups (asthma and rhinitis), there was no significant difference in the level of IL-13 in BALF (p>0.05) (Figure 2E).

Eosinophil Peroxidase Activity

The EPO activity significantly increased in non-treated rhinitis and asthma groups (0.31±0.02 and 0.22±0.01 OD_{492}, respectively) compared to the healthy group (0.1±0.02 OD_{492}) (p<0.001) and significantly decreased in vitamin A-treated rhinitis and asthma groups (0.15±0.01 and 0.14±0.02 OD_{492}, respectively) compared to non-treated rhinitis and asthma groups (Figure 3A) (p<0.001).

Histamine Level

Histamine level showed significant elevation in non-treated rhinitis and asthma groups compared to the healthy group (p<0.05). Also, histamine levels decreased in vitamin A-treated rhinitis and asthma groups as compared with non-treated rhinitis and asthma groups, but these changes were statistically insignificant (p>0.05) (Figure 3B).

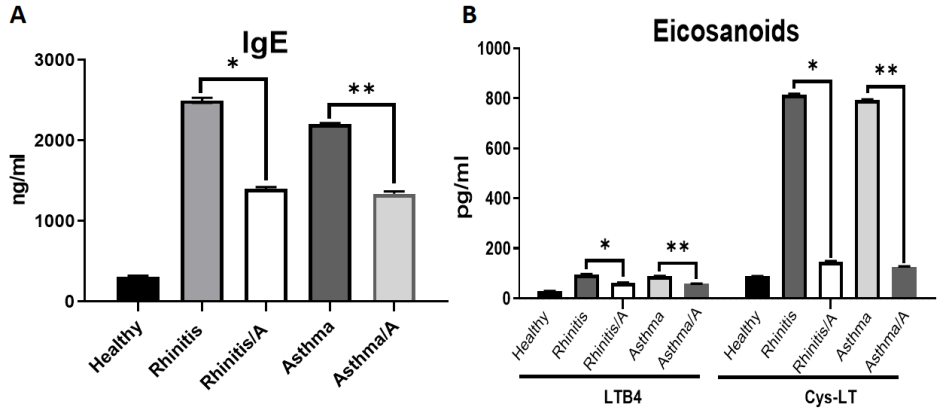

Figure1. Total Immunoglobulin E (IgE), Leukotriene B4 (LTB4), and cysteinyl leukotriene (Cys-LT) levels. In the collected samples, IgE, LTB4, and Cys-LT levels were measured by the enzyme-linked immunosorbent assay (ELISA) method. There were significant differences between vitamin A-treated and non-treated groups. *p<0.05, ** p<0.01

Histopathology

Perivascular and peribronchial inflammation, hyperplasia of the goblet cell, and hyper-secretion of the mucus increased in the non-treated asthma group (3.7±0.2, 3.9±0.1, 3.3±0.5, and 3.5±0.3, respectively) compared with the healthy control group (0.5±0.2, 0.5±0.3, 0.5±0.2, and 0.5±0.3, respectively).

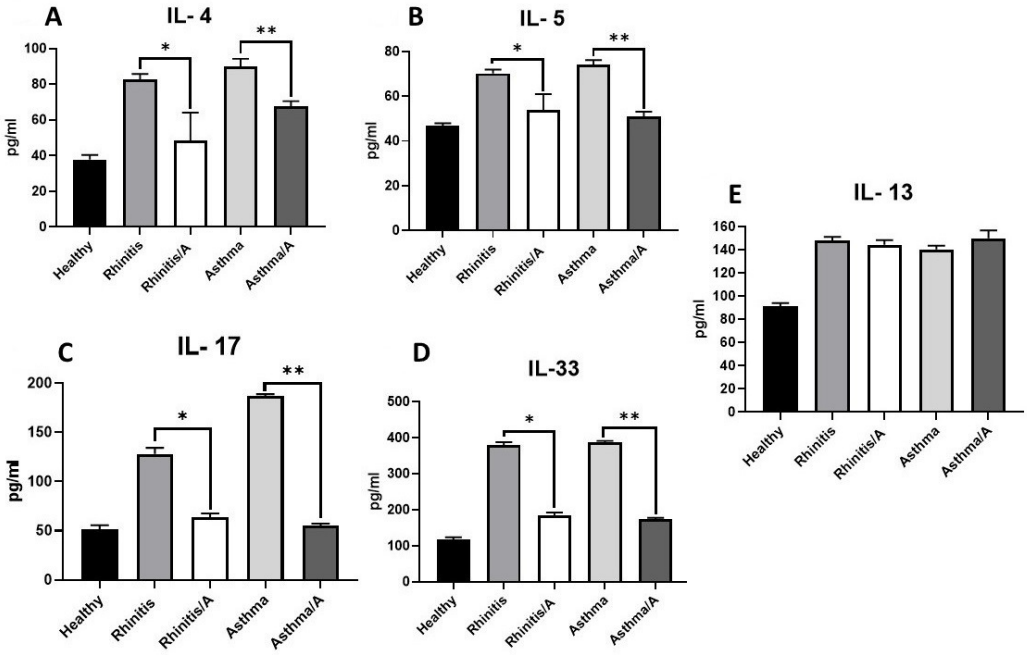

Figure 2. Cytokines levels: The levels of interleukin (IL)-4, IL-5, IL-13, IL-17, and IL-33 in bronchoalveolar lavage fluid (BALF) were measured by the enzyme-linked immunosorbent assay (ELISA) method in the groups that received vitamin A (asthma and allergic rhinitis groups). There were significant differences between vitamin A-treated and non-treated groups comparing IL-4, IL-5, IL-17, and IL-33 levels. *p<0.05, **p<0.01

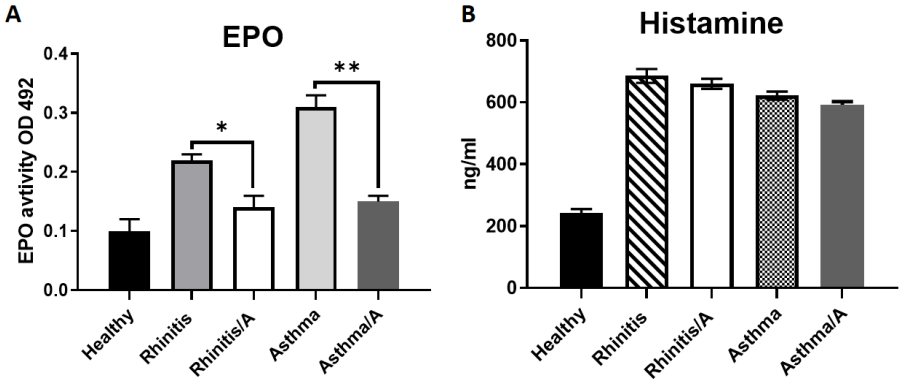

Figure 3. Eosinophil peroxidase activity and histamine level (A). Histamine level and eosinophil peroxidase (EPO) activity in asthma and allergic rhinitis groups (B). There were significant differences between vitamin A-treated and non-treated groups comparing EPO activity. *p<0.05, **p<0.01

Perivascular and peribronchial inflammation significantly decreased in the vitamin A-treated asthma group (2.14±0.4 and 1.92±0.3, respectively) compared to the non-treated asthma group (3.7±0.2 and 3.9±0.1, respectively) (p<0.05) (Figure 4). However, goblet cell hyperplasia and mucus hypersecretion did not reveal any significant decrease in the airways of vitamin A-treated compared with the non-treated asthmatic group (p<0.05) (Figure 4).

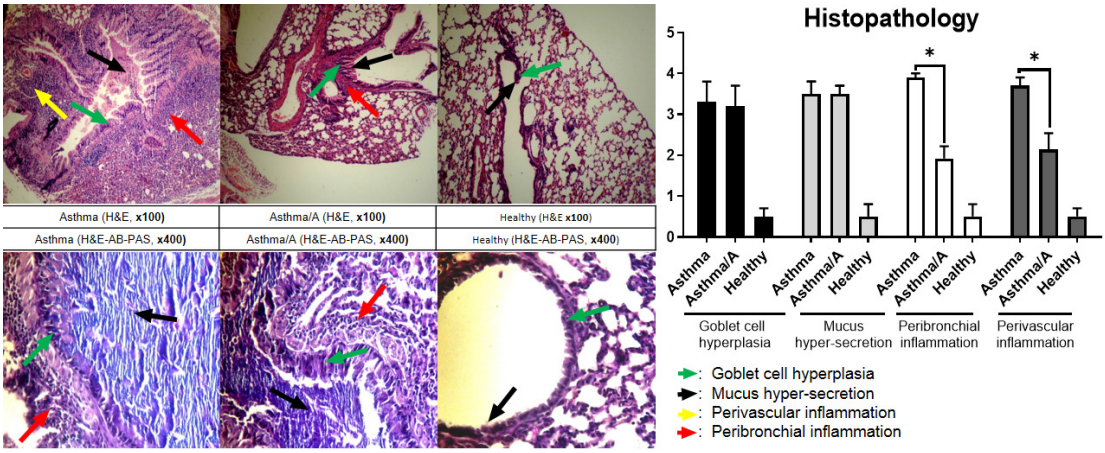

Figure 4. Lung Histopathology. After fixation, lung tissues were stained with Hematoxylin and Eosin (H&E) and Hematoxylin and Eosin-Alcian Blue-Periodic Acid Schiff (H&E-AB-PAS). Afterward, perivascular and peribronchiolar inflammation, goblet cell hyperplasia, and mucus hypersecretion were evaluated. There were significant differences between vitamin A-treated and non-treated groups comparing perivascular and peribronchiolar inflammation. *p<0.05

DISCUSSION

Asthma imposes direct costs such as those for the diagnosis, control, and treatment and also indirect costs caused by inefficient and ineffective work procedures, patients lost time, health system involvement, facilities and equipment used, etc.[3] Controlling asthma attacks are the main goal of asthma treatment. Vitamin A plays an important role in modulating the immunopathologic mechanisms of allergic diseases. In the present study, we found that vitamin A could reduce inflammatory responses in AR and asthma animal models. Vitamin A administration decreased total IgE, LTB4, Cys-LT, IL-4, IL-5, IL-17, and IL-33 levels, as well as EPO activity, which is the main trigger of sex acerbating asthma and allergy symptoms. On the other side, vitamin A had no significant effects on IL-13 and histamine levels, goblet cell hyperplasia, and mucus hyper-secretion.

Vitamin A plays a crucial role in regulating immune system functions, and enough vitamin A maternal intake reduces the risk of allergy in children.[5] Vitamin A and its metabolites after entering the cell, bind to CRBP and then are metabolized and transported by CRABP and/or FABP5 and translocated to the nucleus where they bind to and activate nuclear receptors. Vitamin A binds to nuclear RARs and the PPARβ/δ, leading to the transcription of specific target genes.[10] The etiology of airway hyper responsiveness in asthma is not known, but it has been observed that in vitamin A-deficient rats, muscarinic M-2 receptors played a role in the suppression of bronchoconstriction, and there was a reduction in the expression and function of muscarinic M-2 receptors in bronchial asthma.[10] Also, vitamin A deficiency may also affect airway hyperresponsiveness and bronchial smooth muscle phenotype and differentiation during airway development, probably through regulating TGF-β.[23,24] Increased serum vitamin A in children with stable asthma augmented their quality of life and pulmonary function and protected them against asthma attacks by downregulating oxidative stress indicators and directly modulating the immune system.[25,26]

In our study, we observed that vitamin A treatment decreased allergic factors such as LT-B4, IgE, EPO activity, eosinophilic inflammation, as well as IL-4, IL-5, IL-17, and IL-33 levels in rhinitis and asthma mice models. A study by Elenius et al in 2017 suggested that higher bioavailable vitamin D levels were associated with a lower prevalence of AR; however, higher vitamin A levels showed borderline association with attenuated respiratory problems.[26] On the other hand, in our study, vitamin A could not decrease IL-13 and histamine levels in atopic mice models. Likewise, mucus hyper-secretion and goblet cell hyperplasia were not decreased in vitamin A-treated allergic groups. So, in this study, we showed that vitamin A did not affect IL-13 level and therefore on mucus hyper-secretion. In

the present study, we found that vitamin A could decrease the severity of inflammatory responses; nevertheless, it was not effective in controlling mucus obstruction of airways.

A study showed that higher serum vitamin D levels were associated with a higher expression of IL-37, an anti-inflammatory cytokine that is known to suppress immune responses by inducing tolerance and regulating T-reg development.[27] Lower vitamin A levels were also associated with lower levels of IFN-γ, which may partly explain the association between vitamin A deficiency and asthma exacerbation. Increased levels of IFN-γ, a critical cytokine with multiple functions in the immune system, activate Th1 responses while modulating the Th2 immune branch, which is necessary to control asthma attacks.[28] Vitamin A enhances T-reg activity via FOXP3 and inhibits Th17 development via RORγt signaling pathways.[26]

Lung development is sensitive to changes in the level of vitamin A, and its deficiency leads to lung hypoplasia.[23]Vitamin A-mediated signaling has the main role in the initial steps of airway morphogenesis.[29] Also, vitamin A affects lung smooth muscle development by inducing smooth muscle differentiation-associated genes (*Acta2*, *Csrp1* and *Csrp2*, *heavy chain 11*, *Tagln*, and *Myh11*).[24] Therefore, vitamin A is essential for lung normal physiological activity, and its deficiency can be associated with airway diseases (such as asthma and rhinitis) and interstitial lung disorders(such as COPD and emphysema). We observed that vitamin A received animal models of asthma and AR had lower rates of airway inflammation as compared with non-treated groups.

Asthmatic children have been shown to have lower vitamin A levels than healthy counterparts,[23] and disease severity was reported to highly correlate with serum vitamin A level. Also, the severity of vitamin A deficiency is linked with the severity of wheezing in infants.[30] A study showed that chronic maternal vitamin A deficiency harmed the postnatal lung function of offspring, evidenced by reduced FEV1 and FVC during childhood; these effects could be prevented by proper vitamin A supplementation during gestation.[14]

IL-33 is required for allergen-induced airway inflammation. IL-33 activates Myd88-dependent signaling pathways in the cells expressing the ST2/IL-1RAcP receptor complex, including Th 2 cells. IL-5 and IL-13 are secreted in response to IL-33 and play crucial roles in type-2 allergic responses and eosinophil inflammation.[31,32] IL-33/ST2 signaling is also required for IL-5 and 13 productions in the lung.[33] The genes encoding *IL-33* and *ST2/IL1RL1* were suggested as major susceptibility loci for asthma predisposition in several genome-wide association studies. Interestingly, *IL33* and *ST2/ILRL1* are the two genes reproducibly found to be associated with asthma.[34-36] In our study, IL-33 levels decreased in vitamin A-treated asthma and rhinitis groups compared to non-treated groups. So, vitamin A plays an important role in controlling upper hand cytokine genes (such as *IL-33*) that are important mediators in asthma exacerbation. We found that vitamin A could be a useful supplement to manage allergic rhinitis and asthma symptoms, partly via regulating IL-33.

Vitamin A can interfere with the expression and activation of a variety of genes and signaling pathways in multiple cell types of the lung and airways. This vitamin plays a crucial role in the modulation of type-2 immune responses following exposure to allergens and this manner influences susceptibility to asthma. Thus, future studies are suggested to focus on developing strategies to incorporate vitamin A as a therapeutic agent in allergic diseases such as asthma and AR.

There were some limitations in this study. We did not use other forms or additional doses of vitamin A in this study. We also did not evaluate the IFN-γ level, and therefore, we could not determine the IFN-γ/IL-4 ratio. Also, in this study, airway hyperresponsiveness was not evaluated, and immunohistochemical staining of histopathological sections was not performed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

None

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