Research Article

A Novel Strategy for the Treatment of Allergic Rhinitis: Regulating Treg/Th17 and Th1/Th2 Balance In Vivo by Vitamin D

Baowei Li,1 Xiaoli Zhang,1 Zhezhe Sun,1 Bingxin Xu,2 Jihua Wu,3 Hongdan Liu,1 Haolun Han,1 Lei Wang,1 and Wei Wu1

1Department of Otolaryngology, Strategic Support Forces Medical Center, Beijing 100101, China
2Division Two, Strategic Support Forces Medical Center, Beijing 100101, China
3Department of Pathology, Strategic Support Forces Medical Center, Beijing 100101, China

Correspondence should be addressed to Wei Wu; 1531020050@xzyz.edu.cn

Received 26 May 2022; Revised 24 June 2022; Accepted 30 June 2022; Published 31 July 2022

Academic Editor: Gang Chen

Copyright © 2022 Baowei Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. This prospective study is aimed at observing the number of nasal itching and sneezing in rats from the macroscopic level and examine the pathological changes of nasal mucosa, Th1 and Th2-related cytokines, and Treg/Th17 by vitamin D3 administration from the microscopic level, in order to explore the role of vitamin D in allergic rhinitis and to provide theoretical guidance for prevention and treatment.

Results. There were significant differences in nasal itching and sneezing between the administration groups and the positive groups. Meanwhile, the level of Th1 and Treg in the administration groups increased, while the level of Th2 and Th17 decreased, indicating that the balance of Th1/Th2 was corrected. Our study revealed that vitamin D3 has preventive and therapeutic effects on allergic rhinitis, which provides theoretical guidance for practical application.

1. Introduction

It is conservatively estimated that allergic rhinitis (AR) affects more than 500 million people worldwide and has become a health problem affecting humans [1]. Allergic rhinitis is a noninfectious inflammation that includes a group of symptoms, mainly affecting the nasal mucosa [2]. The pathogenesis of allergic rhinitis is not very clear. At present, Th1/Th2 imbalance is considered to be the main cause, and there is no fundamental treatment plan for allergic rhinitis [3–6].

Immunotherapy is considered to be the standard treatment method for long-life relief of symptoms of allergic rhinitis. Recent studies have identified vitamin D as a potential immunomodulator, and it may affect the outcomes of treatment [7–11]. Vitamin D can affect T cells, B cells, monocytes, and macrophages and regulate the activity of DCs. Also, vitamin D can inhibit the differentiation and maturation of DCs in monocytes and downregulate the expression of related stimulatory molecules, thus reducing T cell activity and producing immune tolerance. Vitamin D deficiency can lead to the occurrence of TH2-biased allergic diseases, which may be related to the imbalance of Treg/Th17 cells.

Some studies have shown that the incidence of severe vitamin D deficiency in patients with allergic rhinitis is significantly higher than that normal people, and some studies have shown that children with allergic rhinitis can reduce the symptoms or score of allergic rhinitis by vitamin D-assisted treatment during pollen season. Although there has been some controversy over the efficacy of vitamin D, recent studies have shown a beneficial effect of vitamin D on the course of allergic disease [12–14]. However, many of those studies focused on the symptoms of allergic rhinitis, but the mechanism of vitamin D in curing allergic rhinitis remains unclear.

This study intended to observe the pathological changes of nasal mucosa, Th1 and Th2-related cytokines, and Treg/Th17 changes in sensitized mice through nasal drops and oral administration of vitamin D, so as to explore the causes...
of vitamin D in the treatment of allergic rhinitis from the micromechanism level and provide theoretical guidance for prevention and treatment.

2. Materials and Methods

2.1. Materials. Ovalbumin, PMA, and 1A,245-dihydroxyvitamin D3 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aluminum hydroxide gel, FOXP3 monoclonal antibody, and IL-17 monoclonal antibody were purchased from Thermo Fisher Scientific (Shanghai, China). CD4 monoclonal antibody and CD 25 monoclonal antibody were purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Experimental Animals. 46 male BALB/c mice weighted 18-20 g in SPF grade were purchased from PLA Strategic Support Characteristic Medical Center. They were randomly divided into 5 groups according to different treatment methods, and each group was randomly divided into two groups once again. All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of PLA Strategic Support Characteristic Medical Center.

2.3. Treatment Methods. The mouse allergic rhinitis model was prepared by ovalbumin and aluminum hydroxide [15, 16].

(a) Suspension preparation: suspension was formed by vibrating mixture of ovalbumin and aluminum hydroxide gel on the oscillator for two minutes. The dose for each mouse is 20 μg ovalbumin in 300 μL aluminum hydroxide gel

(b) Sensitization stage: 100 μL of newly configured suspension (a total of 300 μL for each) was injected subcutaneously into the abdomen, the left and right back of each mouse on days 1, 8, and 15

(c) Stimulation stage: since day 22, mice were atomized with 2% OVA solution and inhaled with ultrasonic atomizer for 30 minutes each time for 7 days

(d) Treatment: 6 mice without any treatment were treated as group 1, 6 allergic rhinitis mice without other treatment were treated as group 2, 8 allergic rhinitis mice treated with vitamin D3 intranasally were named as group 3, anther 8 allergic rhinitis mice treated with vitamin D3 by intraperitoneal injection were named as group 4, and 8 allergic rhinitis mice received PBS injection were considered as group 5.

2.4. Nasal Symptoms. The number of sneezes and nasal rubs was counted within 30 minutes of each stimulation since the first stimulation.

2.5. Inflammatory Factor Detection. The blood was collected by using a pyrogen- and endotoxin-free test tube and centrifuged at 3000 rpm for 10 min to separate the serum and red blood cells quickly and carefully. The levels of IL-4, IL-5, IL-10, IgE, and INF-γ in the supernatant were detected by ELISA kit according to the instructions.

2.6. IHC Staining. CD80, CD86, IFN-γ, and IL-10 immuno-reactivity in nasal mucosa of mice were evaluated by HE and IHC staining. First of all, samples were rehydrated through a process of ethanol for 20 min, 90% ethanol for 2-3 min, 80% ethanol for 2-3 min, and 70% ethanol for 2-3 min. Samples were washed with PBS for 3 times then inactivated by peroxidase at room temperature for 10 min. Then, after washed with PBS, samples were blocked for at 37°C for 1 h. Next, samples were incubated with the primary antibody at 37°C for 2 h and the secondary antibody 37°C for 2 h and the second antibody 37°C for 1 h in sequence. DAB and hematoxylin were added for 5 min at room temperature according to the order.

2.7. Electron Microscopy. Samples were fixed by 4% paraformaldehyde and then immersed in glutaraldehyde at 4°C overnight. After that, samples were soaked in 30%, 50%, 75%, and 90% acetone solution for 10 min according to the order and then soaked in acetone for 5 min (repeated 3 times) for dehydration. After sample preparation, they were observed by TEM.

2.8. Statistical Analysis. All data analyses were performed on GraphPad Prism 8.0 software. P < 0.05 was considered as a statistical significance.

3. Results

3.1. Nasal Symptoms. The result of number of nasal itching and sneezing was shown in Table 1. It could be seen that the nasal itching and sneezing number of groups 3 and 4 was significantly different from groups 1, 2, and 5 (P < 0.05), while it was almost the same between groups 3 and 4, indicating that intranasal administration may be as effective as intraperitoneal administration.

3.2. Inflammatory Factor Detection. As shown in Figure 1, IL-4 and IL-5 levels in group 3 and group 4 were almost the same as group 2 and group 5, while IL-10 level in groups 3 and 4 were slightly higher than that in group 2, which may represent inflammatory factors secretion of Th2, which can promote B cell proliferation and differentiation, induce IgE synthesis, and accelerate respiratory tract remodeling. IFN-γ level in group 3 was slightly lower than that in group 5 which indicated that Th1 was slightly downregulated with vitamin D3 administration.

| Group | Nasal itching | Sneezes |
|-------|---------------|---------|
| 1     | 6             | 0.3 ± 0.5 | 0  |
| 2     | 6             | 11.2 ± 2.6 | 6.3 ± 0.8 |
| 3     | 8             | 5.9 ± 0.8  | 3 ± 0.8 |
| 4     | 8             | 6.3 ± 0.7  | 2.6 ± 0.7 |
| 5     | 8             | 12 ± 1.5   | 6.5 ± 0.9 |

Table 1: Nasal symptoms (x ± s).
In addition, Treg and Th17 cell markers were tested, and the results showed that CD25+ FOXP3+ level in groups 3 and 4 was higher than that in group 2 while CD4+ IL-17+ level in group 3 was lower than that in group 2. This result suggested that in the vitamin D3 treatment group, the tendency of cell differentiation to Treg cells...
Figure 2: Continued.
was enhanced, but the ability to differentiate to Th17 cells was weakened.

3.3. IHC Staining. IHC staining showed the intensity of CD-80, CD-86, IFN-γ, and IL-10 proteins in nasal mucosa. As shown in Figure 2, the expression of four proteins in mice nasal mucosa showed no significant difference among different groups.

3.4. Electron Microscopy. The morphological characteristics of nasal mucosa in different groups were examined by TEM. As shown in Figure 3, the number of intracytoplasmic vacuoles in the nasal mucosal cells of group 2 and group 5 was significantly higher than other groups, indicating the presence of inflammatory response. However, they showed no significant difference between group 1, group 3, and group 4, which mean that administration of vitamin D could inhibit allergic rhinitis in mice.

4. Conclusion

Allergic rhinitis is one of the most common pediatric diseases with high incidence worldwide [17–20]. In recent years, the incidence of allergic rhinitis and some respiratory diseases has increased significantly with the aggravation of air pollution and environmental deterioration. Curing allergic rhinitis effectively and safely has attracted more and more attention. To address the problems associated with allergic rhinitis, in this study, we successfully established a model of allergic rhinitis by using a highly sensitive protein, ovalbumin.

Previous studies showed that the level of serum vitamin D (mainly vitamin D 3) in allergic rhinitis patients was generally lower [21–23]. Also, vitamin D was used as immune modulator in a variety of autoimmune diseases [24–26]. However, most previous studies have focused on animal or human macromanifestations to verify the efficacy of vitamin

![Figure 2: IHC staining. (a) Nasal tissues from the normal control group (group 1), positive control group (group 2), intranasal administration group (group 3), intraperitoneal injection group (group 4), and positive interference group (group 5). Statistical analysis of CD-80-positive (a), CD-86-positive (b), IFN-γ-positive (c), and IL-10-positive (d) staining using ImageJ.](image)

![Figure 3: Transmission electron microscopy (TEM) images of nasal mucosa.](image)
D in the treatment of allergic rhinitis, while the mechanisms have not been thoroughly studied. This may result in limited use of vitamin D. Therefore, in this study, we tested the role of vitamin D3 administration in the development of allergic rhinitis. Patients with allergic rhinitis have increased levels of various proinflammatory factors which also promote disease progression [27, 28]. Our research showed that from the apparent monitoring data, the number of nasal itching and sneezing of groups 3 and 4 (vitamin D3 administration groups) was significantly lower than from groups 2 and 5 (allergic rhinitis group and positive interference group). From a microscopic point of view, the levels of inflammatory factors were assessed. In the vitamin D3 group, the level of IL-10 in groups 3 and 4 was slightly higher than group 2, while the level of IFN-γ in group 3 was slightly lower than group 5, indicating that the level of Th1 was increased and the level of Th2 was decreased, resulting in the Th1/Th2-balance corrected [29–32]. Also, in vitamin D3 treatment groups, the tendency of cell differentiation to Treg cells was enhanced, but the ability to differentiate to Th17 cells was weakened [33–35]. Meanwhile, the results of TEM showed that the nasal mucosa of mice in the inflammatory groups showed obvious inflammatory response, while there was no significant difference between the vitamin D3 administration groups and the control group. Although we used two ways of administration in this article, the experimental results showed that there was little difference in the efficacy of oral administration or intraperitoneal injection. Therefore, in our future possible studies, we may focus more on the mechanism behind the efficacy.

In conclusion, vitamin D has been verified to have effect on allergic rhinitis, which can reduce inflammation.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Baowei Li and Xiaoli Zhang have contributed equally to this work and share first authorship.

References

[1] D. Zhu, Y. Feng, and B. Wang, “Research progress on the relevance between serum vitamin D and IL-33/ST2 levels and allergic rhinitis,” *Journal of Clinical Otorhinolaryngology Head and Neck Surgery*, vol. 33, no. 9, pp. 898–900, 2019.

[2] H. Alnori, F. A. Allassaf, M. Alfahad, M. E. Qazzaz, M. Jasim, and M. N. Abed, “Vitamin D and immunoglobulin E status in allergic rhinitis patients compared to healthy people,” *Journal of Medicine and Life*, vol. 13, no. 4, 2021.

[3] T. Brown, “Diagnosis and management of allergic rhinitis in children,” *Pediatric Annals*, vol. 48, no. 12, pp. e485–e488, 2019.

[4] A. O. Eifan and S. R. Durham, “Pathogenesis of rhinitis,” *Clinical & Experimental Allergy*, vol. 46, no. 9, pp. 1139–1151, 2016.

[5] N. T. Orban, M. R. Jacobson, K. T. Nouri-Aria, S. R. Durham, and A. O. Eifan, “Repetitive nasal allergen challenge in allergic rhinitis: priming and Th2-type inflammation but no evidence of remodelling,” *Clinical & Experimental Allergy*, vol. 51, no. 2, pp. 329–338, 2021.

[6] J. Dong, O. Xu, J. Wang, C. Shan, and X. Ren, “Luteolin ameliorates inflammation and Th1/Th2 imbalance via regulating the TLR4/NF-κB pathway in allergic rhinitis rats,” *Immunopharmacology and Immunotoxicology*, vol. 43, no. 3, pp. 319–327, 2021.

[7] Y. H. Kim, K. W. Kim, M. J. Kim et al., “Vitamin D levels in allergic rhinitis: a systematic review and meta-analysis,” *Pediatric Allergy & Immunology*, vol. 27, no. 6, pp. 580–590, 2016.

[8] N. A. Akbar and M. A. Zacharek, “Vitamin D: immunomodulation of asthma, allergic rhinitis, and chronic rhinosinusitis,” *Current Opinion in Otolaryngology & Head & Neck Surgery*, vol. 19, no. 3, pp. 224–228, 2011.

[9] W. Zhang and Y. Xu, “Association between vitamin D receptor gene polymorphism rs228570 and allergic rhinitis,” *Pharmacogenomics and Personalized Medicine*, vol. 13, pp. 327–335, 2020.

[10] K. Saad, A. Abdelmoghny, M. D. Aboul-Khair et al., “Vitamin D status in Egyptian children with allergic rhinitis,” *Eur. Nose & Throat Journal*, vol. 99, no. 8, pp. 508–512, 2020.

[11] S. Agarwal, S. N. Singh, R. Kumar, and R. Sehra, “Vitamin D: a modulator of allergic rhinitis,” *Indian Journal of Otolaryngology and Head & Neck Surgery*, vol. 71, Supplement 3, pp. 2225–2230, 2019.

[12] S. F. Ansari, M. Memon, N. Brohi, and B. Kumar, “Vitamin D and serum immunoglobulin E levels in allergic rhinitis: a case-control study from Pakistan,” *Cureus*, vol. 11, no. 12, article e6495, 2019.

[13] Y. H. Shin, E. K. Ha, J. H. Kim et al., “Serum vitamin D level is associated with smell dysfunction independently of aeroallergen sensitization, nasal obstruction, and the presence of allergic rhinitis in children,” *Pediatric Allergy and Immunology*, vol. 32, no. 1, pp. 116–123, 2021.

[14] M. Bakhshaee, M. Shariatpanahi, N. M. Arbab, B. Rasoulian, and M. Mohebbi, “Therapeutic effect of vitamin D supplementation on allergic rhinitis,” *European Archives of Oto-Rhino-Laryngology*, vol. 276, no. 10, pp. 2797–2801, 2019.

[15] X. Fang, Q. Xie, and X. Zhang, “Serum vitamin D level in mice with allergic rhinitis is correlated with inflammatory factors,” *American Journal of Translational Research*, vol. 13, no. 4, pp. 3351–3356, 2021.

[16] A. Kempinska-Podhorodecka, M. Milkiewicz, U. Wasik et al., “Decreased expression of vitamin D receptor affects an immune response in primary biliary cholangitis via the VDR-miRNA155-SOCS1 pathway,” *International Journal of Molecular Sciences*, vol. 18, no. 2, p. 289, 2017.

[17] T. B. Won, S. H. Quan, C. S. Rhee, Y. G. Min, and C. Hee Lee, “Expression of uteroglobin in a murine model of allergic rhinitis,” *Acta Oto-Laryngologica. Supplemetum*, vol. 127, no. -sup558, pp. 83–89, 2007.
[18] N. U. Awan, S. K. Sohail, F. Naumeri et al., “Association of serum vitamin D and immunoglobulin E levels with severity of allergic rhinitis,” *Cureus*, vol. 12, no. 1, article e12911, 2021.

[19] D.-D. Zhu, X.-W. Zhu, X.-D. Jiang, and Z. Dong, “Thymic stromal lymphopoietin expression is increased in nasal epithelial cells of patients with mugwort pollen sensitive-seasonal allergic rhinitis,” *中华医学杂志* (Chinese Journal of Medicine), vol. 122, no. 19, p. 2303, 2009.

[20] “Recent advances in allergic rhinitis,” *Journal of Clinical Otorhinolaryngology Head and Neck Surgery*, vol. 29, no. 3, pp. 202–206, 2015.

[21] C. F. Schuler IV and J. M. Montejo, “Allergic rhinitis in children and adolescents,” *Immunology and Allergy Clinics of North America*, vol. 41, no. 4, pp. 613–625, 2021.

[22] W. Gong, Y. Feng, P. Yan et al., “Effect of nasal instillation of vitamin D3 on patient with allergic rhinitis symptoms,” *Journal of Clinical Otorhinolaryngology Head and Neck Surgery*, vol. 28, no. 14, pp. 1031–1033, 2014.

[23] S. J. Lee, B. H. Kang, and B. S. Choi, “Vitamin D serum levels in children with allergic and vasomotor rhinitis,” *Korean Journal of Pediatrics*, vol. 58, no. 9, p. 325, 2015.

[24] J. J. Yepes-Núñez, J. L. Brożek, A. Fiocchi et al., “Vitamin D supplementation in primary allergy prevention: systematic review of randomized and non-randomized studies,” *Allergy*, vol. 73, no. 1, pp. 37–49, 2018.

[25] S. Tao, H. Zhang, Q. Zhao, H. Bu, H. Wang, and H. Guo, “Correlation of vitamin D with inflammatory factors, oxidative stress and T cell subsets in patients with autoimmune hepatitis,” *Experimental and Therapeutic Medicine*, vol. 19, no. 5, pp. 3419–3424, 2020.

[26] A. Broyde, U. Arad, N. Madar-Balakirski et al., “Longterm efficacy of an antipneumococcal polysaccharide vaccine among patients with autoimmune inflammatory rheumatic diseases,” *Journal of Rheumatology*, vol. 43, no. 2, pp. 267–272, 2016.

[27] K. Shimada, M. Gotoh, K. Okubo, T. Hiroi, O. Kaminuma, and A. Nakaya, “Serum cytokine interactions are implicated in the mechanism of action of sublingual immunotherapy for Japanese cedar pollenosis,” *Journal of Nippon Medical School*, vol. 85, no. 5, pp. 250–258, 2018.

[28] C. M. Liu, X. M. Ren, X. Yin et al., “Effects of specific immunotherapy on the expression levels of serum IL-17, IL-35 and Treg/Th17 regulatory T cells in patients with allergic rhinitis caused by dermatophagoides,” *Journal of Clinical Otorhinolaryngology Head & Neck Surgery*, vol. 30, no. 17, pp. 1372–1375;1380, 2016.

[29] B. Smolkova, J. Tulinska, L. Palkovicova Murinova et al., “Impact of interleukin 13 (IL13) genetic polymorphism Arg130Gln on total serum immunoglobulin (IgE) levels and interferon (IFN)–γ gene expression,” *Clinical & Experimental Immunology*, vol. 188, no. 1, pp. 45–52, 2017.

[30] A. Davoodi, R. Lotfi, S. H. Mortazavi, A. G. Karaji, A. Rezaeiemanshe, and F. Salari, “Retinoic acid correlates with reduced serum IL-10 and TGF-β in allergic rhinitis,” *Reports of Biochemistry and Molecular Biology*, vol. 9, no. 4, pp. 399–407, 2021.

[31] Z. Wang and F. Tan, “The blockade of PD-1/PD-L1 pathway promotes the apoptosis of CD19+CD25+ Bregs and suppresses the secretion of IL-10 in patients with allergic rhinitis,” *Scandinavian Journal of Immunology*, vol. 91, no. 2, p. e12836, 2020.

[32] M. G. Kang, S. W. Han, H. R. Kang, S. J. Hong, D. H. Kim, and J. H. Choi, “Probiotic NVP-1703 alleviates allergic rhinitis by inducing IL-10 expression: a four-week clinical trial,” *Nutrients*, vol. 12, no. 5, p. 1427, 2020.

[33] T. Van Nguyen, C. H. Piao, Y. J. Fan et al., “Anti-allergic rhinitis activity of α-lipoic acid via balancing Th17/Treg expression and enhancing Nrf2/HO-1 pathway signaling,” *Scientific Reports*, vol. 10, no. 1, article 12528, 2020.

[34] D. Sheha, L. El-Korashi, A. M. AbdAllah, M. M. El Begermy, D. M. Elzoghby, and A. Elmahdi, “Lipid profile and IL-17A in allergic rhinitis: correlation with disease severity and quality of life,” *Journal of Asthma and Allergy*, vol. 14, pp. 109–117, 2021.

[35] K. Erkan, M. K. Bozkurt, H. Artaç et al., “The role of regulatory T cells in allergic rhinitis and their correlation with IL-10, IL-17 and neopterin levels in serum and nasal lavage fluid,” *European Archives of Oto-Rhino-Laryngology*, vol. 277, no. 4, pp. 1109–1114, 2020.