Observational Study

Medicine®

OPEN

Roles of T follicular helper cells in the pathogenesis of adenoidal hypertrophy combined with secretory otitis media

Chunyan Feng, MS[a], Qicheng Zhang, MS[b], Guangquan Zhou, MS[c], Jing Zhang, MS[a], Yanshu Zhang, MS[c,*]

Abstract

The aim of this study was to investigate the roles of T follicular helper (Tfh) cells in secretory otitis media (SOM) combined with adenoidal hypertrophy (AH).

Patients with AH or AH combined with SOM admitted to the Yancheng No. 1 People's Hospital from December 2012 to December 2014 were included. Fourteen age-matched healthy individuals received physical examinations in the hospital served as control. The venous Tfh was determined using flow cytometry, and CD3 + CD4 + CXCR5 + T lymphocytes were defined as Tfh cells. Serum inflammatory factors including IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70, IL-21, and IgE were determined using commercial kits.

Compared with the AH group, the number of CD4 + CXCR5 + T cells in peripheral blood of the AH combined with SOM group showed significant increase. Statistical differences were noticed in the number of the number of CD4 + CXCR5 + T cells in moderate and severe AH groups compared with that of the control group. Statistical differences were identified in the proportion of CD4 + CXCR5 + T cells in the adenoidal tissues between the AH combined with SOM group and AH group ($P < .05$). For the CD4 + CXCR5 + T cells in adenoidal tissues, no statistical differences were noticed between the moderate and severe AH groups ($P > .05$). The number of CD4 + CXCR5 + T cells was positively correlated to the serum IL-21. Nevertheless, no correlation was noticed between CD4 + CXCR5 + T cell and serum IL-8, IL-6, IL-10, and IgE.

Tfh is involved in the AH combined with SOM in children. Besides, serum IL-21, IL-8, IL-6, IL-10, and IgE may be involved in the onset of SOM in children.

Abbreviations: AH = adenoidal hypertrophy, SOM = secretory otitis media, Tfh = T follicular helper.

Keywords: adenoidal hypertrophy, secretory otitis media, SOM, T follicular helper

1. Introduction

Secretory otitis media (SOM), a common disease in children, is a type of nonpurulent inflammation featured by decreased hearing and presence of middle-ear effusion affecting the life quality of children worldwide.[1] The incidence of SOM in adolescence is significantly higher than that of the adults, which is a major cause for hearing loss in these population.[2,3] Moreover, it may affect the speech, psychological behavior, and mental development.

T follicular helper (Tfh) cells, a CD4 + expressing T-lymphocyte subset, is responsible for assisting B lymphocytes to generate antibodies in humoral immune response.[4] These cells could interact with the corresponding ligands on the surface of B lymphocytes, which contributed to the secretion of IL-21 and IL-4.[5,6] Subsequently, it could involve in the immune response by triggering the proliferation and differentiation of B lymphocytes, as well as the class switching of immune globulin.[7]

SOM in children involved various factors, especially chronic tonsillitis and adenoidal hypertrophy. Meanwhile, aberrant Tfh expression has been frequently reported in those with autoimmune diseases including sjogren syndrome, systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, and myasthenia gravis.[8,9] Moreover, Tfh cells may implicate in the pathogenesis of these diseases as the autoantibody is positively correlated with the Tfh proportion and pathogenicity.[10] Under pathological states such as adenoiditis, adenoidal hypertrophy (AH) and tonsillitis, adenoid was subject to stimulation of various spoilage organisms such as local bacterial virus, and then presented mucous hyperemia, lymphadenosis, as well as activation and proliferation of Tfh.[6] Meanwhile, Tfh could be access to the pharyngotympanic tube and the mucous membrane of middle-ear space via blood circulation,[11] which then contributed to the release of inflammatory mediators leading to SOM.[12]

Nowadays, increasing evidence indicates that adenoid immune dysfunction may induce and even aggravate SOM, but the exact mechanism is still not well defined.[13] In this study, we aim to investigate the roles of Tfh in the pathogenesis of SOM combined

Editor: Satyabrata Pany.

CF and QZ equally contributed to this work.

The authors have no conflicts of interest to disclose.

[a] Department of Hematology, Yancheng City No. 1 People's Hospital, Yancheng, [b] Department of Otorhinolaryngology Head and Neck Surgery, Affiliated Hospital of Nantong University, Nantong, [c] Department of Otorhinolaryngology Head and Neck Surgery, Yancheng City No. 1 People's Hospital, Yancheng, China.

[*] Correspondence: Yanshu Zhang, Department of Otorhinolaryngology Head and Neck Surgery, Yancheng City No. 1 People's Hospital, Yancheng 224005, China (e-mail: zysycent@163.com).

Medicine (2018) 97:13(e0211)

Received: 26 August 2017 / Received in final form: 9 January 2018 / Accepted: 28 February 2018

http://dx.doi.org/10.1097/MD.0000000000010211

with AH. Our study contributed to the early screening and treatment of SOM combined with AH.

2. Materials and methods

2.1. Patients

Patients with AH or AH combined with SOM admitted to the Yancheng No. 1 People's Hospital from December 2012 to December 2014 were included in this study. Meanwhile, 14 age-matched healthy individuals received physical examinations in the hospital were included as normal control. SOM was diagnosed based on auripuncture combined with acoustic immitance, pure tone test, and endotoscope. The diagnosis of AH and chronic tonsillitis was based on the pathological findings, together with lateral projection of the nasopharynx and pharyngorhinoscopy. Each subject signed the informed consent. The study protocols were approved by the Ethical Committee of Yancheng City No. 1 People's Hospital.

The severity of AH was categorized according to the evaluation of adenoid based on the nasal endoscopy:[14] mild hypertrophy, obstruction of posterior nares of < 1/3; moderate hypertrophy, obstruction of posterior nares of 1/3 to 2/3; severe hypertrophy, obstruction of posterior nares of > 2/3.

2.2. ELISA

Venous blood (4 mL) were collected from each patient, and the serum was obtained after centrifugation at 2500 r/min for 5 minutes at 4°C. Serum IL-21 and IgE were determined using commercial ELISA kits purchased from Beyotime (Shanghai, China), according to the manufacter's instructions. All tests were performed at least in triplicate.

2.3. Flow cytometry

Venous Tfh was determined using flow cytometry. Briefly, 2 mL venous blood was collected from each patient. Afterward, corresponding antibodies including FITC-CD3, PE-Cy7 -CD4, and APC-CXCR5 were added, followed by incubation at room temperature for 20 minutes. After resuspending with PBS, the samples were subjected to flow cytometry. For the level of Tfh in adenoidal tissues, the fresh adenoidal tissues were washed using PBS 3 times after crashing into pieces using the scissors. Then the samples were cultured in RPMI 1640 medium, and the resuspension was subjected to 300 mesh filter. Subsequently, the suspension was subjected to filter and resuspended in 1640 medium. The mixture was centrifuged at 800 g at room temperature for 5 minutes. CD3 + CD4 + CXCR5 + T lymphocytes were defined as Tfh cells (Fig. 1).

2.4. Determination of serum inflammatory factors

Serum inflammatory factors (e.g., IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70, IL-21, and IgE) were determined using commercial kits (BD OptEIA ELISA sets, BD Science, CA). The tests were performed at least in triplicate.

2.5. Statistical analysis

SPSS17.0 software was used for the data analysis. All data were presented as mean ± standard deviation. Student's *t*-test was used for the intergroup comparison of venous blood sample and the adenoidal samples. $P < .05$ was considered to be statistically significant.

3. Results

3.1. Patient characteristics

Twenty-three cases (male: 15, female: 8, median age: 6.0 years) with AH combined with SOM, 26 cases (male: 14, female: 12, median age: 7.0 years) with AH and 14 normal control (male: 8, female: 6, median age: 7.0 years) were included in this study. No statistical differences were noticed in the clinical features among 3 groups ($P > .05$).

3.2. Elevation of CD4 + CXCR5 + T cells in AH patients

Statistical increase was noticed in the CD4 + CXCR5 + T cells in peripheral blood in the AH combined with SOM group compared with AH group and control group ($P < .05$, Fig. 2). Compared with AH group, the number of CD4 + CXCR5 + T cells in peripheral blood of the adenoidal hypertrophy combined with SOM group showed significant increase (Fig. 2A). Besides, compared with the control group, the number of CD4 + CXCR5 + T cells in peripheral blood of the AH group showed statistical increase ($P < .05$).

3.3. Comparison of CD4 + CXCR5 + T cells in moderate and severe AH and normal control

In this part, we compared the number of CD4 + CXCR5 + T cells in moderate and severe AH groups and normal control. Statistical increase was noticed in the number of CD4 + CXCR5 + T cells in moderate and severe AH groups compared with that of the control group ($P < .05$, Fig. 2B). The number of CD4 + CXCR5 + T cells in moderate AH group showed no significant difference compared with that of the severe AH group ($P > .05$).

3.4. Comparison of CD4 + CXCR5 + T cells in adenoidal tissues

Student's *t*-test was used to investigate the proportion of CD4 + CXCR5 + T cells in the adenoidal tissues between the AH combined with SOM group and AH group. The results showed statistical differences were identified in the proportion of CD4 + CXCR5 + T cells in the adenoidal tissues between the AH combined with SOM group and AH group ($P < .05$, Fig. 3A). For the CD4 + CXCR5 + T cells in adenoidal tissues, no statistical differences were noticed between the moderate and severe AH groups ($P > .05$, Fig. 3B).

3.5. Comparison of serum IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70, IL-21, and IgE

The serum IL-8 in the AH combined with SOM group showed significant increase compared with that in the control group and AH group, respectively ($P < .05$, Fig. 4A). However, no statistical differences were noticed between the control group and AH group ($P > .05$). The serum IL-1b showed no statistical differences among the AH combined with SOM group, AH, and control group ($P > .05$, Fig. 4B). The serum IL-6 in the AH combined with SOM group was significantly lower than that of the control group and AH group, respectively ($P < .05$, Fig. 4C). However, no statistical differences were noticed in the serum IL-10 between the control group and AH group ($P > .05$). The serum TNF and IL-12p70 showed no statistical differences among the AH combined with SOM group, AH group, and control group ($P > .05$, Fig. 4D and E). The serum

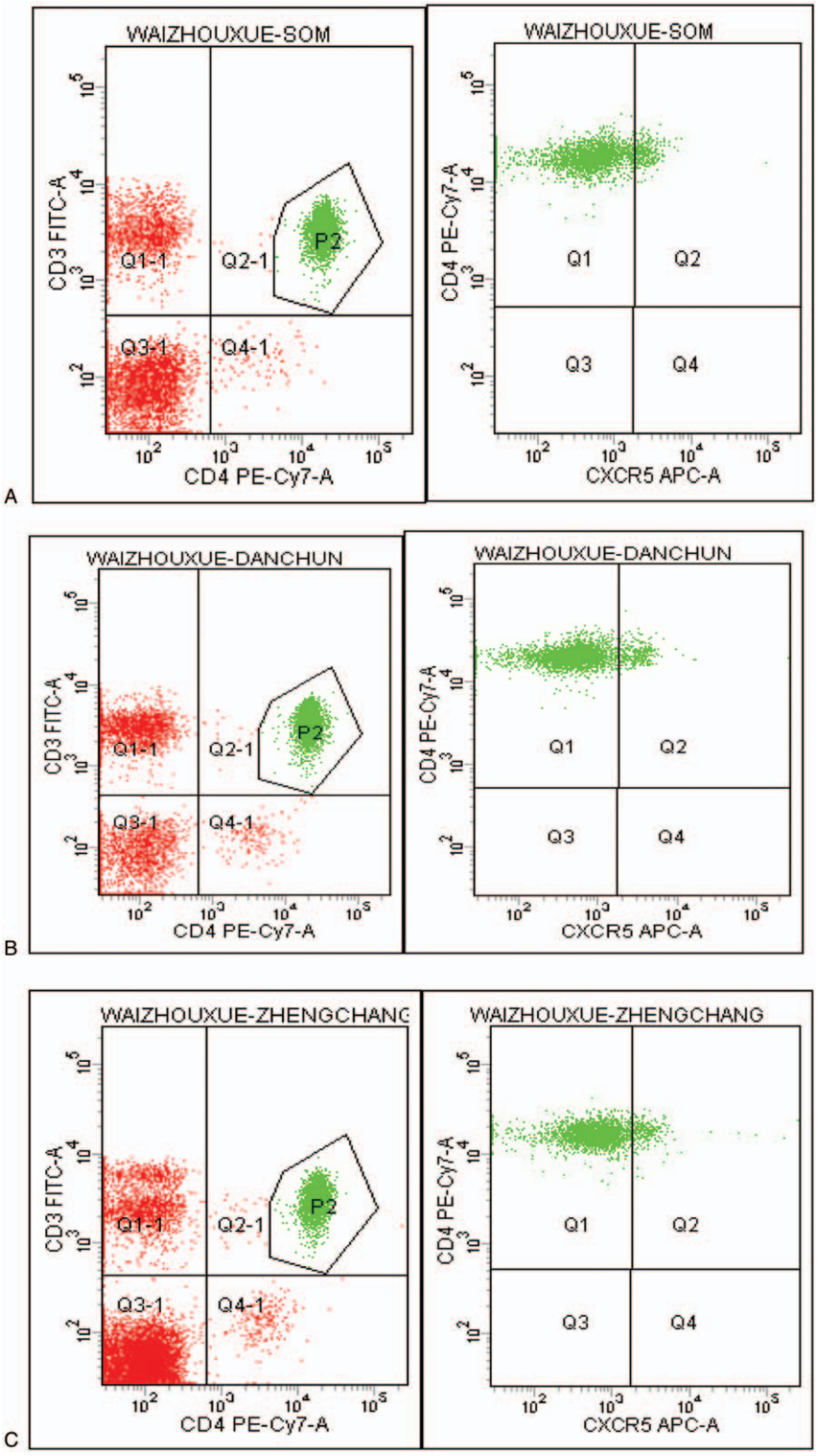

Figure 1. Expression of CD3FITC-A and CD4PE-Cy7-A in patients with SOM combined with AH (A), AH (B), and control (C). AH = adenoidal hypertrophy, SOM = secretory otitis media.

IL-21 in AH combined with SOM group was significantly higher than that of the AH group ($P < .05$, Fig. 4F), while the serum IL-21 in AH group was significantly higher that of control group ($P < .05$, Fig. 4G). Serum IgE in the AH combined with SOM group was significantly higher than that of AH group ($P < .05$, Fig. 4H). No statistical differences were noticed in the IgE between the AH group and control group ($P > .05$).

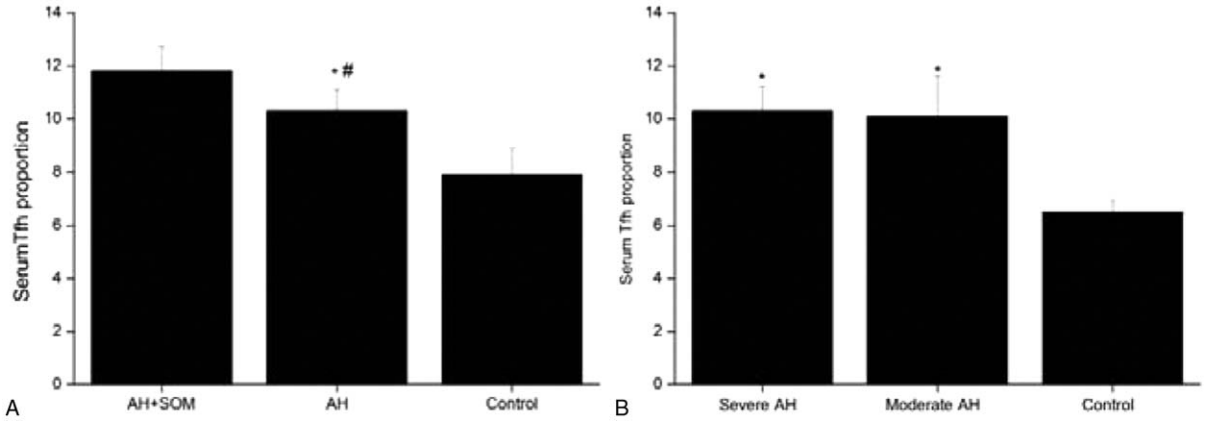

Figure 2. Comparison of serum Tfh proportion. (A) Comparison of Tfh proportion between AH combined with SOM group, AH group, and control group. $^{*}P < .05$ versus AH + SOM group; $^{\#}P < .05$ versus control group. (B). Comparison of Tfh proportion patients with severe AH, moderate AH, and control. $^{*}P < .05$ versus control group. AH = adenoidal hypertrophy, SOM = secretory otitis media, Tfh = T follicular helper.

3.6. Correlation between CD4 + CXCR5 + T cell proportion and serum IL-21, IL-8, IL-6, IL-10 and IgE

Correlation analysis was performed to investigate the correlation between CD4 + CXCR5 + T cell proportion and serum IL-21, IL-8, IL-6, IL-10, and IgE. The number of CD4 + CXCR5 + T cells was positively correlated to the serum IL-21. Nevertheless, no correlation was noticed between CD4 + CXCR5 + T cell and serum IL-8, IL-6, IL-10, and IgE ($P > .05$, Fig. 5).

3.7. Serum IL-21 and Tfh after treatment

AH patients were then subject to adenoidectomy, and then the serum IL-21 and Tfh was determined. The results showed that the CD4 + CXCR5 + T cell proportion and serum IL-21 showed significant decrease after adenoidectomy compared with the baseline level ($P < .05$, Fig. 6) in those with AH with or without SOM.

4. Discussion

The pathogenesis of SOM is considered to be related to the interaction between infection, immune imbalance, and auditory tube dysfunction. In childhood, AH has been reported as a major cause for the SOM as it may induce disorder in the drainage of auditory tube and middle-ear cavity.[15] Besides, auditory tube reflux may be usually presented in those with AH as these patients always concurrent with chronic rhinitis and nasal obstruction, which then finally induced SOM. Moreover, patients with AH were susceptible to retrograde infection in auditory tube. In a previous study, Gates et al[16] reported that the volume of adenoid showed no direct correlation to the onset of SOM, and the pathogenesis of SOM was somehow related to the immune dysfunction of the adenoids.

As an important immune organ, adenoid contains various cells that involve in the immune response including T cells, B cells, plasmocytes, dendritic cells, and phagocytes. According to the previous study,[17] patients with SOM showed paraplasia of mastocytes in the adenoids, which triggered the massive release of interleukins, platelet chemokines, and histamine and the subsequent local inflammation, mucosal edema and formation of middle-ear effusion. Kiroglu et al[18] revealed that SOM patient with adenoid subjected to long-term exposure to bacteria and virus contributed to the onset of immune response and the formation of middle-ear effusion. Besides the paraplastic immunocytes, the cytokines released by immunocytes also played important roles in the pathogenesis of SOM. Moreover, aberrant changes of cytokines were noticed in those with SOM, and obvious changes were noted in the SOM patients with different severity and ages for the same cytokine.[19] All these confirmed that abnormalities in the adenoid immune function may involve in the pathogenesis and development of SOM.

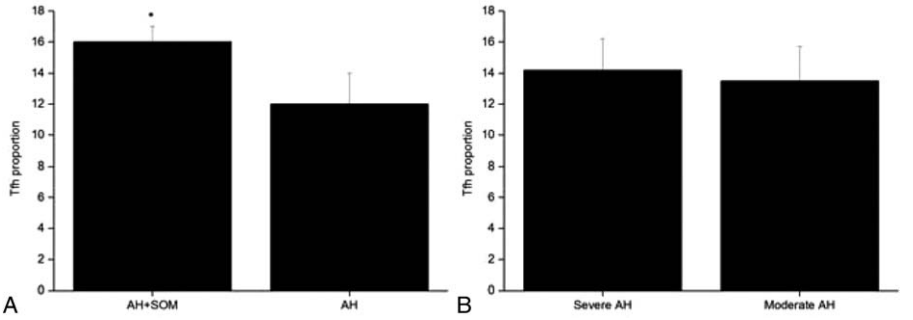

Figure 3. Proportion of CD4 + CXCR5 + T cells. (A) Proportion of CD4 + CXCR5 + T cells in the adenoidal tissues between the AH combined with SOM group and AH group. $^{*}P < .05$ versus AH group. (B) Proportion of CD4 + CXCR5 + T cells in severe AH group and moderate AH. AH = adenoidal hypertrophy, SOM = secretory otitis media.

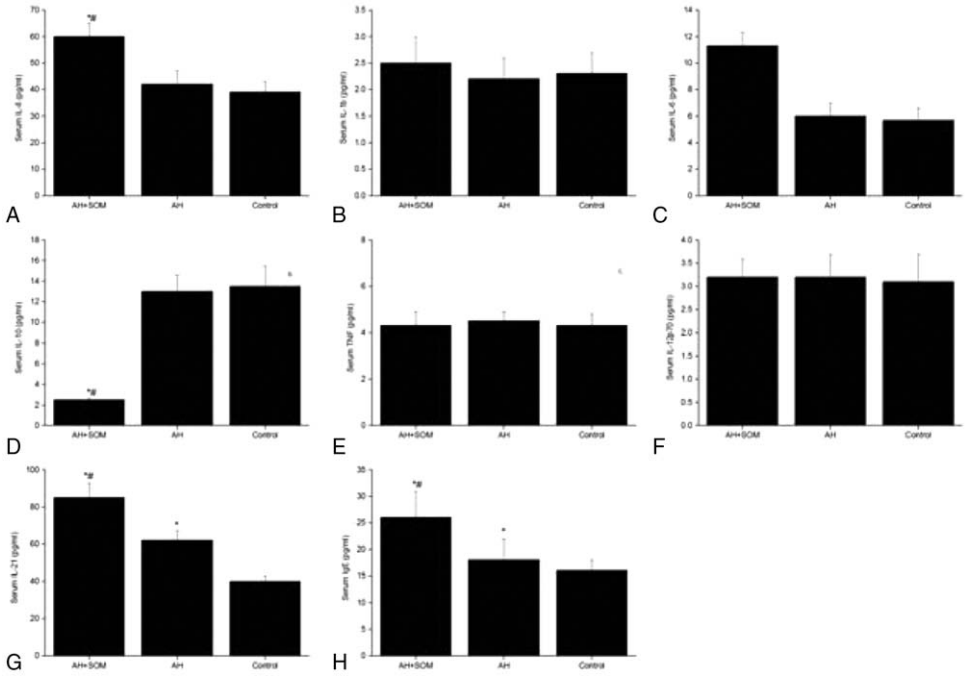

Figure 4. Comparison of serum IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70, IL-21, and IgE between the AH combined with SOM group and AH group. $^{\#}P < .05$ versus AH group; $^{*}P < .05$ versus control group. AH = adenoidal hypertrophy, SOM = secretory otitis media.

CXCR5, a major surface marker for Tfh cells, is a chemokine receptor of the Tfh as it could modulate the migration of Tfh to germinal center and interact with the B lymphocytes through ICOS and CD40L, which subsequently contributed to the proliferation and differentiation of B lymphocytes, as well as the secretion of cytokines. IL-21, playing an important role in the Tfh biological activity, could interact with the IL-21R to maintain the proliferation, survival, and differentiation of B lymphocytes.[20–22] Additionally, IL-21R is also expressed on the surface of Tfh cells. Upon the disruption of the IL-21 and IL-21R, the Tfh cell count would be decreased sharply.[23] In this study, we selected CD4 + CXCR5 + T lymphocytes as Tfh cells, and compared the Tfh level through analyzing the proportion of CD4 + CXCR5 + T lymphocytes to the CD4 + cells.

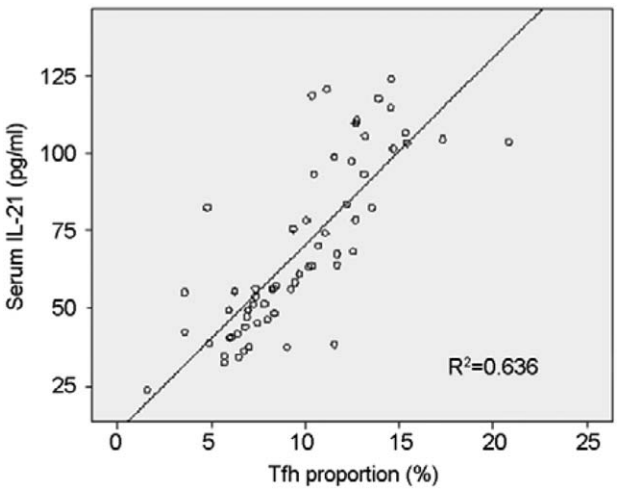

Figure 5. Correlation analysis between serum IL-21 and Tfh proportion. Tfh = T follicular helper.

Recently, studies on Tfh have been focusing on its roles in the autoimmune diseases, including primary Sjogren's syndrome,[24] systemic lupus erythematosus,[25] and rheumatoid arthritis.[26] Increasing evidence showed that Tfh was closely related to the onset of various diseases, such as X-linked lymph proliferative disease[27] and lymphoma; however, rare studies have focused on the roles of Tfh cells in children with AH combined with SOM. In this study, we determined the Tfh level in the peripheral venous blood and the adenoidal tissues using flow cytometry in patients with AH combined with SOM and the normal control. The results showed that the Tfh in venous blood was significantly higher in the AH combined with SOM compared to the healthy control. In addition, patients with AH combined with SOM showed higher Tfh level compared to those with AH only, which demonstrated that aberrant elevation of Tfh in the peripheral venous blood in those with AH combined with SOM. On this basis, it is reasonable to speculate that aberrant proliferation of Tfh may play an important role in the SOM. For the correlation between Tfh and AH, patients with severe and moderate AH showed significantly higher in the Tfh compared with the normal control. however, no statistical differences were noticed in the Tfh in those with severe AH compared to those with moderate AH ($P > .05$). Similarly, no statistical differences were identified in the Tfh in the adenoid tissues in those with severe AH compared to those with moderate AH. These suggested that Tfh was associated with aberrant proliferation of Tfh; however, it showed no correlation with the severity of AH.

In this study, the serum cytokines secreted by Tfh including IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70, IL-21, and IgE were determined. In those with AH combined with SOM, the serum IL-21, IL-8, IL-6, and IgE showed significant increase compared with those in the patients with AH or normal control ($P < .05$).

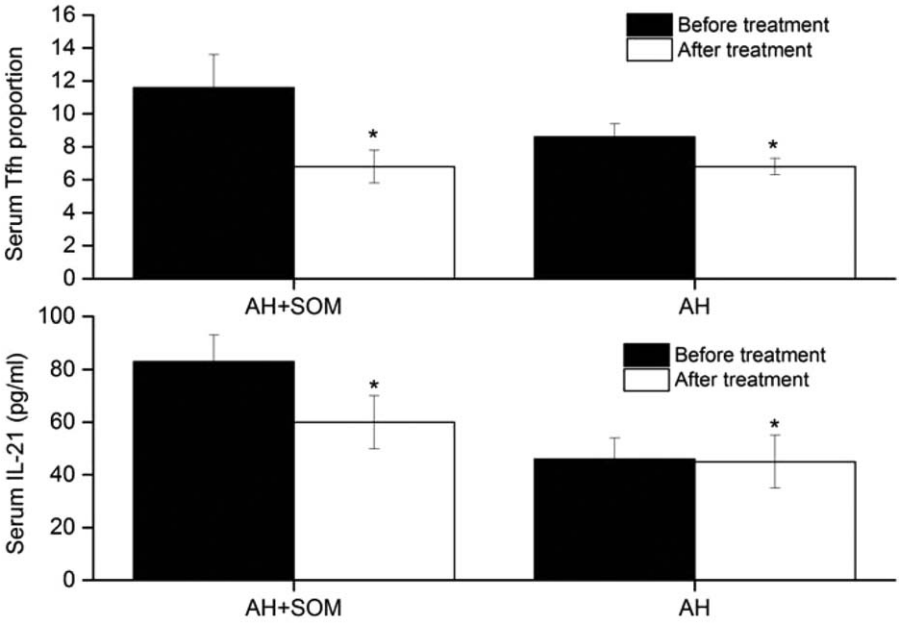

Figure 6. Comparison of serum IL-21 and Tfh after treatment. $^{*}P < .05$. Tfh = T follicular helper.

This indicated that these cytokines may involve in the SOM in children. Besides, the pathogenesis of AH combined with SOM may be closely related to the imbalance of immune modulation of the adenoid. Interestingly, statistical differences were noticed in the IL-21 in the AH patients compared with the normal control, which implied that IL-21 may be closely related to the pathogenesis of AH combined with SOM. In our study, we determined the correlation between Tfh and IL-21 after adenoidectomy, which showed that the proportion of Tfh and IL-21 showed significant decrease after treatment in those with AH with or without SOM. This confirmed that Tfh may involve in the development of AH combined with SOM.

In conclusion, we determined the proportion of Tfh cells and the effect factors in the pathogenesis of AH combined with SOM. Our data showed that Tfh involved in the AH combined with SOM in children. Besides, serum IL-21, IL-8, IL-6, IL-10, and IgE may involve in the onset of SOM in children.

Author contributions

Conceptualization: Q. Zhang.
Data curation: Q. Zhang.
Formal analysis: G. Zhou.
Funding acquisition: G. Zhou.
Investigation: J. Zhang.
Methodology: J. Zhang.
Writing – original draft: C. Feng.
Writing – review & editing: Y. Zhang.

References

[1] Parlea E, Georgescu M, Calarasu R. Tympanometry as a predictor factor in the evolution of otitis media with effusion. J Med Life 2012;5:452–4.
[2] Qureishi A, Lee Y, Belfield K, et al. Update on otitis media—prevention and treatment. Infect Drug Resist 2014;7:15–24.
[3] Kitamura K, Iino Y, Kamide Y, et al. Clinical practice guidelines for the diagnosis and management of acute otitis media (AOM) in children in Japan —2013 update. Auris Nasus Larynx 2015;42: 99–106.
[4] Chiodi F, Bekele Y, Lantto Graham R, et al. IL-7 and CD4 T follicular helper cells in HIV-1 infection. Front Immunol 2017;8:451.
[5] Li Q, Liu Z, Dang E, et al. Follicular helper T Cells (Tfh) and IL-21 involvement in the pathogenesis of bullous pemphigoid. PLoS One 2013;8:e68145.
[6] Shim GJ, Kis LL, Warner M, et al. Autoimmune glomerulonephritis with spontaneous formation of splenic germinal centers in mice lacking the estrogen receptor alpha gene. Proc Natl Acad Sci U S A 2004;101:1720–4.
[7] King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. Annu Rev Immunol 2008;26:741–66.
[8] Szabo K, Papp G, Barath S, et al. Follicular helper T cells may play an important role in the severity of primary Sjogren's syndrome. Clin Immunol 2013;147:95–104.
[9] Simpson N, Gatenby PA, Wilson A, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum 2010;62:234–44.
[10] Ma J, Zhu C, Ma B, et al. Increased frequency of circulating follicular helper T cells in patients with rheumatoid arthritis. Clin Dev Immunol 2012;2012:827480.
[11] Ars B, Dirckx J. Eustachian Tube Function. Otolaryngol Clin North Am 2016;49:1121–33.
[12] Hirano T, Kodama S, Kawano T, et al. Accumulation of regulatory T cells and chronic inflammation in the middle ear in a mouse model of chronic otitis media with effusion induced by combined eustachian tube blockage and nontypeable haemophilus influenzae infection. Infect Immun 2015;84:356–64.
[13] Zelazowska-Rutkowska B, Wysocka J, Ratomski K, et al. Increased percentage of T cells with the expression of CD127 and CD132 in hypertrophic adenoid in children with otitis media with effusion. Eur Arch Otorhinolaryngol 2012;269:1821–5.
[14] Kurien M, Lepcha A, Mathew J, et al. X-Rays in the evaluation of adenoid hypertrophy: its role in the endoscopic era. Indian J Otolaryngol Head Neck Surg 2005;57:45–7.
[15] Takahashi H, Hayashi M, Honjo I. Compliance of the eustachian tube in patients with otitis media with effusion. Am J Otolaryngol 1987;8:154–6.
[16] Gates GA, Muntz HR, Gaylis B. Adenoidectomy and otitis media. Ann Otol Rhinol Laryngol Suppl 1992;155:24–32.
[17] Berger G, Ophir D. Possible role of adenoid mast cells in the pathogenesis of secretory otitis media. Ann Otol Rhinol Laryngol 1994;103(8 Pt 1):632–5.
[18] Kiroglu MM, Ozbilgin K, Aydogan B, et al. Adenoids and otitis media with effusion: a morphological study. Am J Otolaryngol 1998;19: 244–50.

[19] Post JC. Direct evidence of bacterial biofilms in otitis media. 2001. Laryngoscope 2015;125:2003–14.
[20] Yu D, Rao S, Tsai LM, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity 2009;31:457–68.
[21] Linterman MA, Beaton L, Yu D, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med 2010;207:353–63.
[22] Ozaki K, Spolski R, Ettinger R, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol 2004;173:5361–71.
[23] Nurieva RI, Chung Y, Hwang D, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 2008;29:138–49.
[24] Schmutz C, Hulme A, Burman A, et al. Chemokine receptors in the rheumatoid synovium: upregulation of CXCR5. Arthritis Res Ther 2005;7:R217–229.
[25] Linterman MA, Rigby RJ, Wong RK, et al. Follicular helper T cells are required for systemic autoimmunity. J Exp Med 2009;206:561–76.
[26] Jin L, Yu D, Li X, et al. CD4+CXCR5+follicular helper T cells in salivary gland promote B cells maturation in patients with primary Sjogren's syndrome. Int J Clin Exp Pathol 2014;7:1988–96.
[27] Ma CS, Hare NJ, Nichols KE, et al. Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+T cells. J Clin Invest 2005;115:1049–59.