The single-cell landscape of tonsil lymphocytes in pediatric tonsil hyperplasia and obstructive sleep apnea

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Conclusion

Tonsils from children with large tonsils and severe sleep apnea showed a reduced frequency of a subtype of ILC3 (NRP1+ LTi-like cells) and higher frequency of naïve B-cells. The enrichment of naïve B cells could not be explained by cellular proliferation. The causative mechanism of these findings could be a defective differentiation and/or migration in the germinal center. Our study contribute to an increased understanding of immune alterations in pediatric OSA tonsils.

Background

Tonsil hyperplasia is the most common cause of pediatric obstructive sleep apnea (OSA). Despite the growing knowledge in tissue immunology of tonsils, the immunopathology driving tonsil hyperplasia and OSA remains unknown.

Material and Methods

We used multi-parametric flow cytometry to analyze the composition and phenotype of tonsillar T cells, innate lymphoid cells (ILCs; ILC1, ILC2, ILC3 including lymphoid-tissue inducer (LTi) cells) as well as B cells from pediatric patients with OSA, who had previous polysomnography. Guided by unbiased clustering analysis to delineate lymphocyte heterogeneity (data not shown), we used conventional flow cytometric gating to compare cell type frequencies between two patient groups: significantly enlarged tonsils with very severe OSA (n=13), and as controls small tonsils with less severe OSA (n = 6). See table 1 for patient cohort description. Statistical significance was calculated using Mann-Whitney U test.

Parameter	Small tonsils (n = 6)	Large tonsils (n = 13)	p
Age at operation, mean (SD), months	38 (8)	34 (8)	0.4
Sex, No. (%)			
Male	2 (33)	8 (38)	
Female	4 (67)	5 (62)	
Height, mean (SD), cm	94 (7)[a]	92(5)	0.9
Weight, mean (SD), kg	14 (2)[a]	14 (3)	0.9
BMI z-score, mean (SD)	-0.7 (2.0)[a]	-0.4 (1.8)	0.6
Tonsil size[b], median (IQR)	2 (2–2.5)	4 (4–4)	<0.001
OAHI, median (IQR), events/hour of sleep	9.5 (7–11)	35 (33–36)	<0.001

Abbreviations: ATE, adenotonsillectomy; OAHI, Obstructive Apnea-Hypopnea Index.

[a] One missing value in the group with small tonsils (n = 5).

[b] Tonsil size scored according to Brodsky (scored according to occlusion (%) of the oropharynx: 1 = 0–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%).

p-value calculated using Mann-Whitney U test

Table 1. Baseline characteristics of the patient cohort

Results

The two groups showed a similar composition of T cells including naïve, memory and follicular helper (T_{FH}) CD4+ cells, naïve and effector CD8+ cells, as well as double negative (DN) and double positive (DP) T cells.

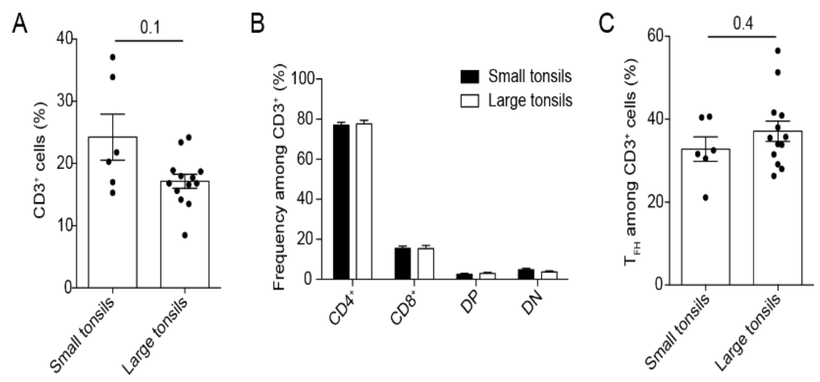

Fig. 1 (A) Frequency of CD3+ T cells among total living CD45+ cells. (B) Frequency of CD4+, CD8+, DP and DN T cells among total CD3+ cells. (C) Frequency of T_{FH} cells among total T cells. Lines, bars and error bars indicate mean ± standard deviation (SD).

Similar frequencies of ILC1 and ILC2 were observed in the groups. A tendency of lower percentage of total ILC3 was seen in large tonsils. The subtype NRP1+ LTi-like ILC3 showed lower frequency among large tonsils.

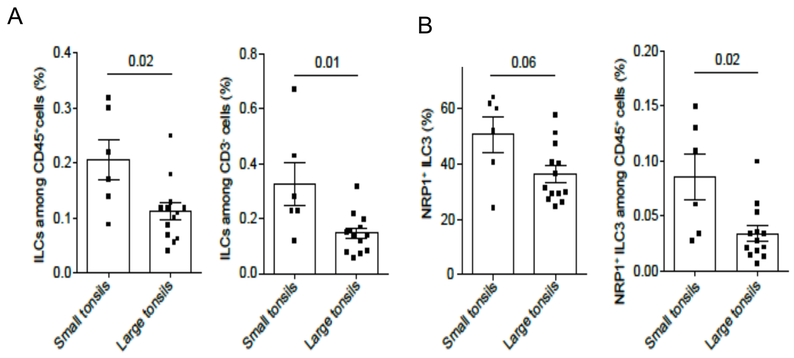

Fig. 2 (A) Frequency of ILCs among total living CD45+ cells and CD3- cells. (B) Frequency of NRP1+ sub-population among ILC3 and total living CD45+ cells.

The large tonsil-group showed an enrichment of CD27-CD21hi naïve B-cells, while the expression of proliferation marker Ki67 was similarly expressed between the groups.

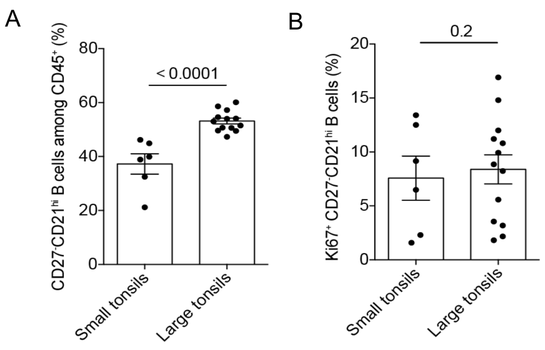

Fig. 3 (A) Frequency of CD27-CD21hi B cells among total living CD45+ cells. (B) Frequency of Ki67+ cells among CD27-CD21hi B cells.

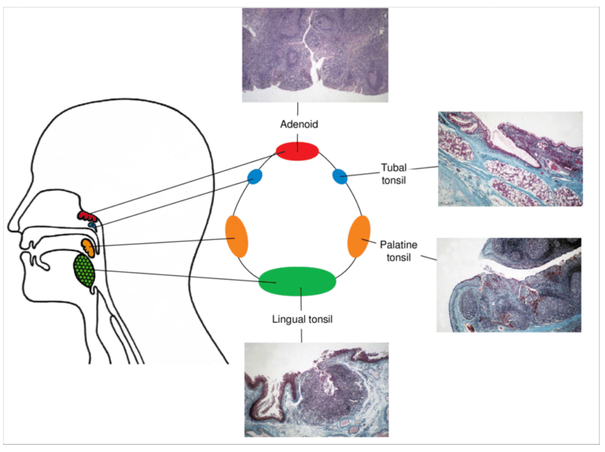

Fig. 4. Waldeyer's ring

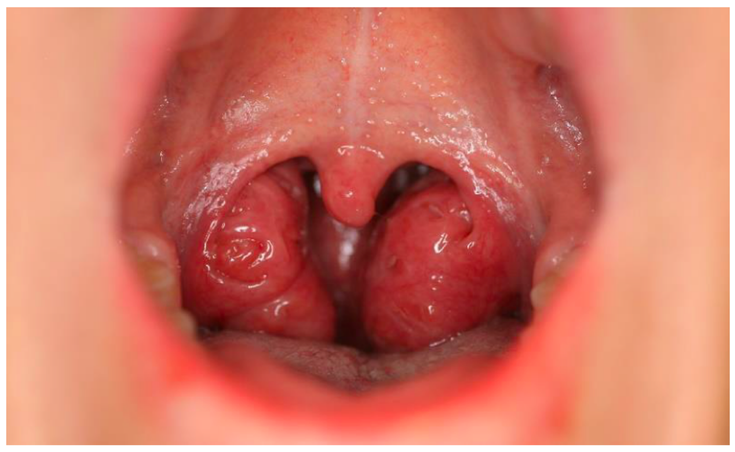

Fig. 5 Tonsil hyperplasia

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